Genetic epidemiological investigation of blood pressure and its metabolic correlates in Mexican American children

by

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Geethavani Chittoor
M.Sc., S.V. University, India, 1998

Submitted to the Department of Anthropology and the Faculty of the Graduate School of the University of Kansas in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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Abstract

Anthropological geneticists have applied methods and theories used in the analyses of normal variation to study complex diseases and their associated risk factors. This dissertation is an investigation of blood pressure (BP) and its correlation with metabolic syndrome (MS)-related traits using a cohort of Mexican American children. The major goals of this dissertation are to: (1) examine various MS-related traits in Mexican American children such as obesity, dyslipidemia, hypertension, impaired fasting glucose, impaired glucose tolerance, insulin resistance, and microalbuminuria; (2) determine the genetic influences on measures of systolic blood pressure (SBP), diastolic blood pressure (DBP) and other variables (e.g., fasting insulin, fasting glucose, microalbuminuria, lipids, and adiposity measures) by estimating heritabilities; (3) examine common genetic influences (i.e., pleiotropy) on BP and its related MS-related traits (e.g., obesity) by using bivariate analyses; and, (4) investigate potential genetic-by-environment (e.g., obesity and lifestyle factors such as physical activity) interaction influences on BP. This study examined 604 nondiabetic Mexican American children and adolescents aged 6-17 years. Variance component analysis is used for both univariate and multivariate models to partition the total phenotypic variation of a given trait(s) into genetic and environmental components. The results indicate high to moderate occurrence of various MS risk factors in these children (overweight = 52%, obesity = 33%, low HDL = 24%, prediabetes = 15%, increased abdominal obesity = 14%, and high blood pressure = 13%). These findings signify the increasing burden of disease risk in these children due to elevated BP, higher prevalence of obesity, and lower insulin sensitivity. The heritabilities of SBP, DBP, and elevated BP are high in this population, suggesting that additive genetic factors strongly influence BP. The relative contributions of both genetic and environmental influence were quantified, genes accounted for 25 - 85% of the phenotypic variation in measures of blood pressure, adiposity, lipids, microalbuminuria, insulin and glucose, whereas age, gender, puberty and physical fitness and other environmental covariates accounted for <45% of the total phenotypic variance. Thus, the clustering of MS-related traits due to pleiotropy in adults is also demonstrable in children of Mexican American ethnic background.

This dissertation is dedicated to the loving memory of my father, Sri Rajasekhara Reddy Chittoor. He was always proud of me and believed in me. He was the one who told me to, "act as you like, do as you like, and live as you like." Whatever I am today is because of him.

Acknowledgments

I would extend my sincere thanks to SAFARI participants for making this project possible. I also appreciate the entire SAFARI team at SFBR, especially Dr. Ravindranath Duggirala, for advice and access to SAFARI data; Dr. Sobha Puppala, for SOLAR training and analysis of data; Dr. Daniel Hale, Ms. Sharon Fowler, Ms. Jennifer Schneider, Ms. Rhonda Lyons, and Mr. Roy Resendez, for assisting me in finishing on time. This research would not have been possible without their help.

In addition, I would like to thank my committee members: Dr. Michael Crawford, Dr. James Mielke, Dr. John Kelly, Dr. Ravindranath Duggirala, and Dr. Kim Kimminau, for their invaluable guidance; my fellow graduate students and friends: Anne Justice, for being my cheerleader and commiserate; Kristine Beaty, Norberto Baldi-Salas, Chris Phillips; Dr. Kris Young, Dr. Rohina Rubicz, Dr. Phil Melton, and Aaron Clark for their continued support and encouragement; the departmental office staff: Judy Ross, Carol Archinal, and Kathleen Womack, for their warmth and concern; my close friend and confidant: Abhinav, for his motivation, optimism, everlasting care and confidence; and my family: Pavan, for letting me pursue higher education; son: Himay, for his smile being my inspiration; mother: Suneetha, for her unconditional love, support and sacrifices; sister-in-law: Swathi, for her cordial nature; and brother: Prasad, for guiding with his firm belief and courage, he is my pillar and I cannot imagine coming this far without him. All of my achievements were possible because of God's grace, these individuals and several other friends and relatives. I am grateful to have each one you as a part of my life.

The data used for fulfilling the specific aims of this study are obtained by an ongoing project at Southwest Foundation for Biomedical Research (SFBR) in San Antonio called the San Antonio Family Assessment of Metabolic Risk Indicators in Youth (SAFARI) Study.

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CHAPTER ONE: INTRODUCTION

Anthropological geneticists study human variation within and between populations to better understand the causes of variation (Reddy *et al.* 1997). Since anthropometric traits vary within and between populations, studies have been conducted to identify the relative contribution of genetic and environmental factors to phenotypic variation (Williams-Blangero and Blangero 1989; Relethford *et al.*1990). Anthropological geneticists have applied methods and theories used in the analyses of normal variation to study complex diseases and their associated risk factors (Majumdar and Rao 1991; Williams-Blangero and Blangero 1993). This dissertation is a genetic epidemiological investigation of blood pressure, a complex phenotype, and its correlation with metabolic syndrome-related traits using a cohort of Mexican American children, in an attempt to understand the web of relationships between obesity and hypertension.

Obesity, both adulthood and childhood, is a growing global public health problem, and childhood obesity is a potential major contributor to the increased prevalence of high blood pressure in children (Torrance *et al*. 2007). According to Virdis *et al*. (2009), it appears that tracking of blood pressure from childhood to adulthood is documented and that the occurrence of obesity in younger ages has a clinical role. Indeed, as described by Bellisari (2008) in anthropological terms, such an increased occurrence of obesity in recent years is the result of interaction between human biology and human culture over a long period of evolution. Obesity and hypertension have strong genetic determinants, the rapid rise in the prevalence rates

of such disease conditions in recent decades is probably attributable to changing environmental factors including socioeconomic and life style factors (e.g., poor dietary habits [high calorie intake] and reduced physical activity levels) and obesogenic environments such as fast food restaurants and increased television viewing time (Harper 2006; Mehta 2007; Gable *et al*. 2007). In such changing environments, it is presumable that "individuals possessing the appropriate combination of ancestral energy-conserving genes (gene sequences regulating energy levels in the body) at greater risk for overweight and obesity and associated chronic diseases" (Bellisari 2008:165).

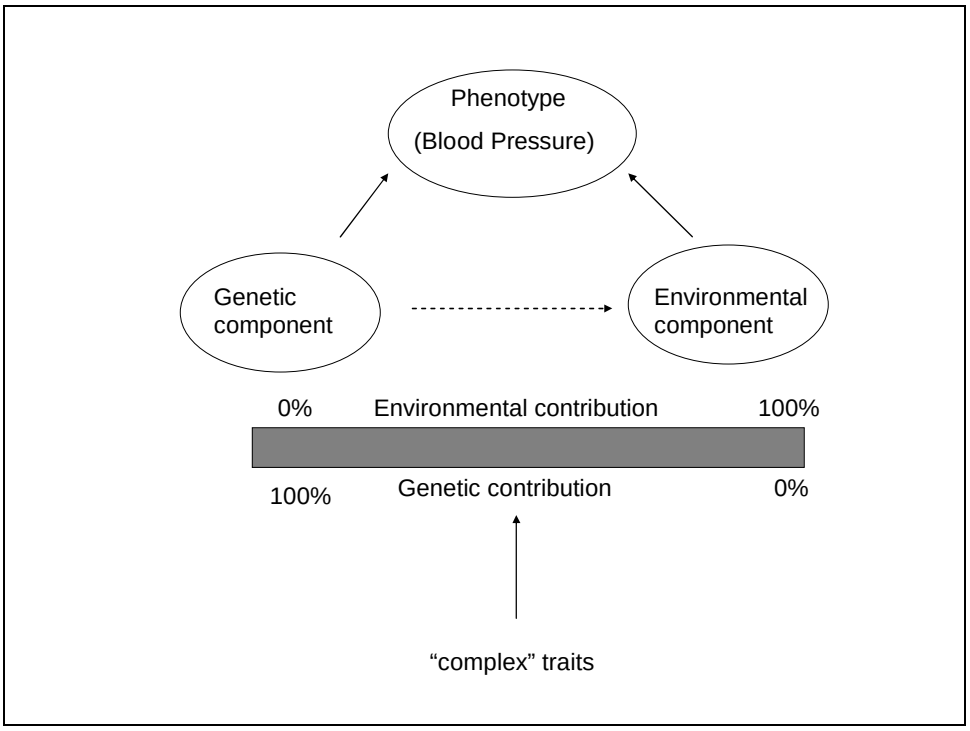

Figure 1: The total phenotypic variation (VP) of a given complex trait (e.g., blood pressure) can be partitioned into genetic (VG) and environmental (VE) components. The proportion of phenotypic variance that is attributed to additive genetic effects (VG/VP) is called heritability (h^2).

The phenotypes (P) such as blood pressure and obesity are complex in nature, which are influenced by genetic (G) and environmental (E) factors and their interactions (IGE) (i.e., P = G + E + IGE; Figure 1). The total phenotypic variation of a given trait (e.g., blood pressure) [after accounting for the influences of environmental covariates (e.g., age and sex), if known] can be partitioned into additive genetic and environmental components, using techniques such as the variance components analysis (Falconer, 1989; Almasy and Blangero, 1998). The covariances or correlations among biological relatives can be used to partition the total phenotypic variance of a complex trait into its genetic and environmental components. The proportion of phenotypic variance that is attributed to (additive) genetic effects is called heritability (h^2) (Figure 1).

In addition to the determination of trait-specific genes (i.e., genes that independently influence variation in a trait such as blood pressure), the genetic basis of correlated traits such as blood pressure and obesity can also be explored using techniques such as variance components analysis (Falconer 1989; Almasy and Blangero 1998; Duggirala *et al*. 2001). In both theory and practice, the pleiotropic effects of genes (see Figure 2) are of particular interest because "the genetic cause of correlation is chiefly pleiotropy", defined as "simply the property of a gene whereby it affects two or more characters, so that if the gene is segregating, it causes simultaneous variation in the characters it affects" (Falconer 1996:312).

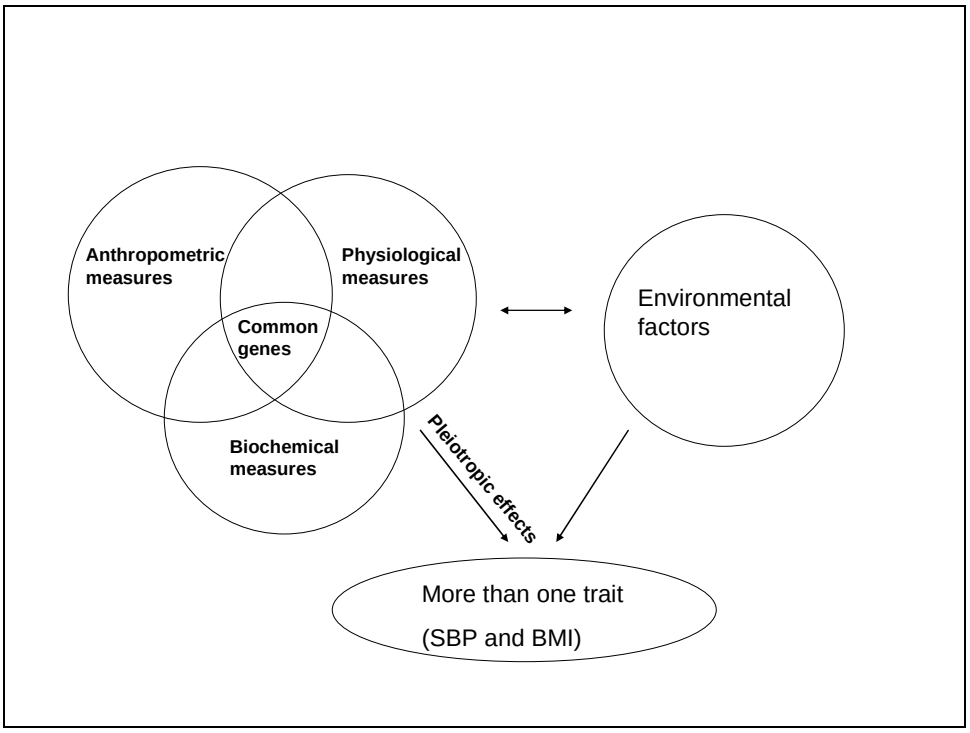

Figure 2: Illustration of common genetic influences (pleiotropy) on correlated traits such as systolic blood pressure and body mass index (BMI) along with environmental effects.

According to World Health Organization (WHO), systolic blood pressure (maximum pressure exerted by the blood on arterial walls [SBP]) at or above 140 mm Hg and diastolic blood pressure (minimum pressure in the blood vessels [DBP]) at or above 90 mm Hg are considered as high/elevated blood pressure [EBP] or hypertension. For example, a SBP of 120 and a DBP of 80 are considered normal and written as 120/80 mm Hg. Blood pressure is a quantitative trait influenced by the complex interaction of genetic and environmental factors illustrated in Figure 1. Studies indicate that blood pressure variation is attributable to a variety of physiological, socio-cultural, and environmental variables (see Figure 2) (Crews and

Williams 1999; Puppala 2001). While no clear explanatory model has been proposed yet (Crews and Williams 1999; Puppala 2001), it has been suggested that the variability in blood pressure within populations is due to several factors such as diet, sodium intake, psychosocial stressors, ecological factors, cultural differences, and underlying pre-dispositions to etiology associated with ethnicity and biology (Sorof and Daniels 2002; Sorof *et al.* 2004). Other factors include familial aggregation, but the contribution of this factor should be interpreted cautiously since shared or common familial environmental influences can mimic shared genetic influences (Duggirala *et al.* 2000b). Although environmental factors, both common familial and individual specific-random environmental influences, affect blood pressure variation, there is ample evidence that blood pressure variation has strong genetic determinants (Rice *et al.* 1989).

As Puppala *et al*. (2009) have recently summarized, several studies reported the heritability of blood pressure and hypertension to be 15-50%. Heritabilities are population specific and can alter during an individual's life span. It is important to note that heritability is a property of not only the variable but also of the population along with the environmental circumstances to which the individuals are exposed, and the manner in which the phenotype is measured (Falconer and Mackay 1996). In recent years, a number of genome-wide linkage scans for blood pressure and hypertension identified several chromosomal regions across the genome that contain susceptibility genes for blood pressure and hypertension (Puppala *et al*. 2009). In addition, very recently, some studies have used a genome-wide association study (GWAS) approach

to localize susceptibility genes for hypertension or blood pressure (e.g., Levy *et al*. 2009; Adeyemo *et al*. 2009).

As stated above, hypertension and obesity are correlated traits. In fact, the term metabolic syndrome (MS) refers to the clustering of various metabolic abnormalities such as obesity, insulin resistance and hyperinsulinemia, impaired fasting glucose, impaired glucose tolerance [after 2-hr glucose load], dyslipidemia (elevated triglyceride and decreased high-density lipoprotein [HDL] cholesterol levels), and hypertension (Reaven 1988; DeFronzo 1995; Kraja *et al.* 2005; Joy and Hegele 2008; Joy *et al.* 2008). Several other phenotypes included as metabolic syndrome components are microalbuminuria [higher levels of albumin/creatinine ratio in the urine (Lee *et al.* 2006)], elevated markers of chronic inflammation, acanthosis nigricans [darkened patches on the skin mostly around neck and under the arms or the groin region because of abnormally acting insulin receptors (Stuart *et al.* 1994, 1997; Burke *et al.* 2000)], endothelial dysfunction, polycystic ovary syndrome, and nonalcoholic fatty liver disease (Henry 2003; Isomaa 2003; Goldstein 2003; Tracy 2003; Cordain *et al.* 2003; Malnick *et al.* 2003; Rowley *et al.* 2003). Metabolic syndrome (MS) is also referred to as the Insulin Resistance Syndrome (IRS) or the Dysmetabolic Syndrome or Syndrome X (Timar *et al.* 2000; Groop and Orho-Melander 2001; Isomaa 2003).

Several diagnostic criteria have been proposed for MS, but the one recommended by the National Cholesterol Education Program [NCEP]/Adult Treatment Panel III [ATPIII] (NCEP/ATPIII 2001) is widely used. According to this definition, metabolic syndrome requires the presence of at least three of five factors: increased

waist circumference (>102 cm in men; >88 cm in women), hypertriglyceredemia (≥150 mg/dl), low HDL cholesterol (<40 mg/dl) in men; <50 mg/dl in women), hypertension (≥130/85 mm Hg), and high fasting glucose (≥110 mg/dl). In recent years, several analytical tools such as bivariate linkage analysis and factor analysis have been employed to localize genes with common genetic influences (pleiotropy) on MS-related traits (Duggirala *et al.* 2001; Arya *et al*. 2002b).

Among the several epidemiological diseases (epidemiology is a "science that deals with the etiology, distribution, and control of disease in groups of relatives, and with inherited causes of diseases in populations" Morton 1978:1), childhood obesity is a strong predictor in the development of various chronic conditions in adulthood such as type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) (Tortolero *et al.* 1997; Berenson 2002; Srinivasan *et al.* 2002; Li *et al.* 2003). Obesity is a condition signifying health of a person based on body composition. There are several ways to measure obesity, and the frequently used technique is by calculating BMI. It is assumed that an individual belongs to a morphological category (obese: BMI $\geq 95^{th}$ percentile for age and sex or overweight: BMI $\geq 85^{th}$ percentile for age and sex among children) if an underlying genetically determined risk or liability exceeds certain threshold, such traits are also known as threshold traits. Childhood obesity and related risk factors result in a set of medical and psychosocial consequences. The medical consequences can be further divided into metabolic conditions that include diabetes mellitus, hypertension, dyslipidemia, non-alcoholic fatty liver disease; and mechanical conditions including obstructive sleep apnea syndrome and orthopedic disorders. The psychological and

social consequences such as discrimination and stigmatization of overweight and obese children also impact their emotional development (Lee 2009). The increasing prevalence of obesity/overweight in children and adolescents is paralleled by increasing prevalence rates of its associated disease conditions including T2DM, MS, hyperlipidemia and hypertension (Spiotta and Luma 2008). Populations such as Mexican Americans are more prone to complex conditions such as obesity (given in Figure 3), T2DM and MS compared to general population of the United States (US). There is evidence for highest age-adjusted prevalence of MS (~32%) in Mexican American adults; and, Mexican American children who are obese or overweight have a greater risk of 20% higher prevalence and exhibit similar metabolic syndrome risk profiles as in adults (Cook *et al.* 2003; de Ferranti *et al.* 2004; Butte *et al.* 2005). Indeed, the important factor associated with metabolic syndrome risk profiles in childhood and adolescence is family history of T2DM. Hence, by examining the children of the adults whose family-based cohorts have individuals with prediabetes (impaired fasting glucose or impaired glucose tolerance or both) and diabetes, we can establish metabolic syndrome profiles as well as understand if there is any distinct etiology in children.

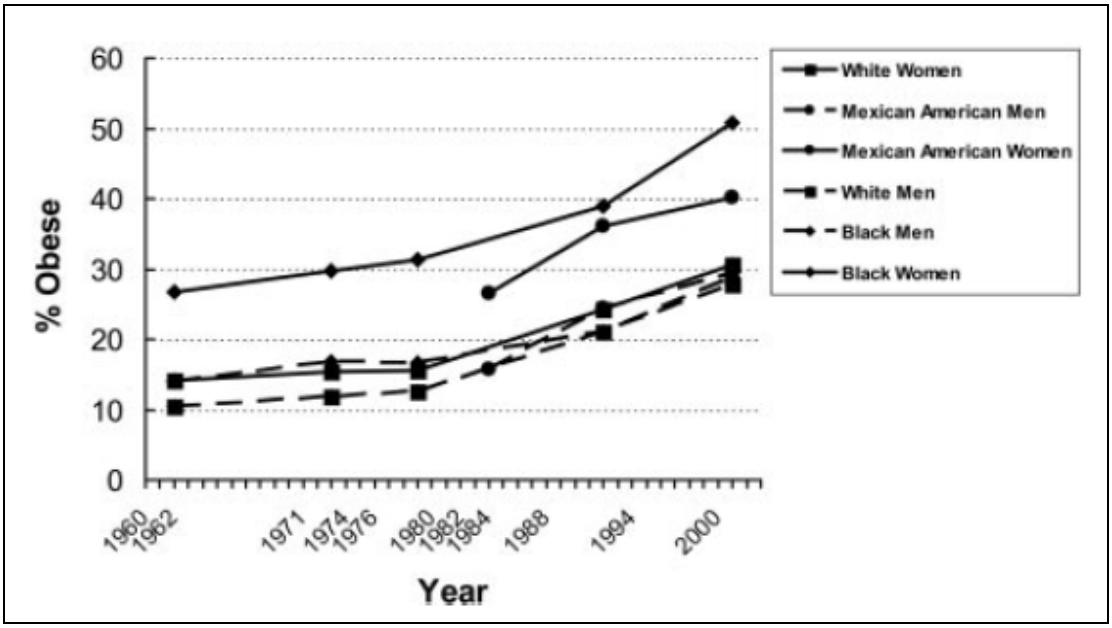

Figure 3: The upward trend of increased obesity (BMI ≥30 kg/m^2) in the US populations by race, ethnicity and gender is illustrated (Smith *et al.* 2005).

The prevalence rates of obesity, hypertension, and T2DM are dramatically increasing and affecting both children and adults in US, as well as in other countries, and such disease patterns demand immediate attention. An emerging public health issue in pediatric populations is the high blood pressure and its correlation with overweight/obesity (Torrance *et al.* 2007), although knowledge of genetic factors that underlie such a correlation is limited. Studies reveal that there is a strong association of body mass index with systolic blood pressure, diastolic blood pressure and also with central obesity [waist circumference >102 cm in men and >88 cm in women; also measured from waist to hip ratio ≤0.8 in women and ≤1.0 in men] (Venkataramana *et al.* 2001; NCEP/ATPIII 2001; Fezeu *et al.* 2007; Ostchega *et al.* 2009;

www.centralobesity.com). These associations are assumed to continue in children, and it will be interesting to determine the factors, both genetic and environmental, that predispose children to these traits. Further, to examine the extent to which they differ with adults in the manifestation of a disease phenotype such as hypertension.

However, it should be noted that the available, but limited, data are not consistent regarding the association between blood pressure and other MS risk factors in children (Steinberger *et al*. 2009). Despite advances in examining the genetic factors that influence variation in blood pressure and its correlated MS-related traits in adults, little is known about the genetic architecture of blood pressure or hypertension and its correlation with various MS-related traits in children and adolescents. Especially in populations of the US such as Mexican Americans who are at high risk for the development of obesity, T2DM, hypertension, and MS. In addition, although the MS definition in adults has been shown to be useful both in clinical and research areas, there are no set criteria to define MS phenotype in children; and, there have been several criteria to define MS in children and adolescents (e.g., Cook *et al.* 2003; Cruz *et al.* 2004; Zimmet *et al.* 2007; Steinberger *et al*. 2009). In consideration of such difficulties, as emphasized in a very recent American Heart Association Scientific Statement (Steinberger *et al*. 2009:1), there is an immediate need "for early detection and preventive measures regarding cardiometabolic risk factors in children and adolescents, with strong focus on obesity, inflammation, insulin resistance, dyslipidemia, and hypertension, which emerge as core elements of morbidity". Such early detection of MS risk factors, including blood pressure and obesity, in children and

adolescents in large and well-characterized cross-sectional pediatric cohorts is very much needed in order to examine such risk factors prospectively, given "the limited data that track individuals from childhood to adulthood" (Steinberger *et al*. 2009:1). Indeed, as the recent American Heart Association Scientific Statement (Steinberger *et al*. 2009:10) remarks, the MS risk factors differ significantly by ethnicity; the MS risk profiles need to be evaluated using criteria that are specific to ethnicity; and, that the genetic and environmental factors that underlie such differential MS risk profiles are poorly understood. Also, as stated previously, there is an immediate need for early detection of MS risk factors including obesity and blood pressure in the pediatric population and for precise understanding of the genetic and environmental influences on such traits, both in univariate and multivariate terms.

Therefore, the major goals of this dissertation are to: (1) examine various metabolic syndrome-related traits in Mexican American children such as obesity, dyslipidemia, hypertension, impaired fasting glucose, impaired glucose tolerance, insulin resistance, and microalbuminuria; (2) determine the genetic influences on measures of systolic blood pressure, diastolic blood pressure and other variables (e.g., fasting insulin, fasting glucose, microalbuminuria, lipids, and adiposity measures) by estimating heritabilities; (3) examine common genetic influences (i.e., pleiotropy) on blood pressure and its related metabolic syndrome-related traits (e.g., obesity) by using bivariate genetic analyses; and, (4) investigate potential genetic-by-environment (e.g., obesity and lifestyle factors such as physical activity) interaction influences on blood pressure. Thus, this dissertation will characterize the pattern of blood pressure variation

in Mexican American children and its association with metabolic syndrome-related phenotypes. Given attention to the differences in components of MS in children by ethnicity, which may be attributable to population genetic backgrounds, it is hypothesized that the variation in blood pressure and its correlated traits is under strong additive genetic influences, and that the additive genetic factors contribute significantly to the association between blood pressure and other MS risk factors in Mexican American children.

CHAPTER TWO: LITERATURE REVIEW

Association between obesity, diabetes and metabolic syndrome

Globally speaking, obesity and T2DM are on the upsurge (nearly epidemic magnitude) and are major concerns in developing as well as developed countries. The WHO estimates the occurrence of diabetes to rise from 171 million in 2000 to 366 million in 2030. India is expected to have about 79 million people afflicted with diabetes by the year 2030, and will be the first (followed by China and the US) of the top 10 countries in the world estimated to have the highest number of people with diabetes (Wild *et al.* 2004). According to the International Obesity Task Force (WHO), about 1.1 billion adults are estimated to be overweight and 312 million are considered to be obese. These are suggestive of an association between obesity and diabetes (Grubbs 2002) and that the link between obesity and T2DM is pronounced (Nadler and Attie 2001; Caterson and Gill 2002).

The prevalence rates of obesity and T2DM differ among populations in the US. Mexican Americans and African Americans are particularly at high risk for the development of these conditions. For example, in Mexican Americans the incidence of T2DM is twofold compared to non-Hispanic Europeans and about ~ 2 million (10%) Mexican Americans have T2DM (Stern *et al.* 1983; Burke *et al.* 1999). Based on the San Antonio Heart Study (SAHS), a secular trend for T2DM was found in Mexican Americans in the 1980s (increased from 5.7% in 1979 to 15.7% in 1988) (Burke *et al.* 1999). For example, in the SAHS which is a population-based longitudinal study to assess T2DM and CVD risk, the results showed that individuals who developed T2DM

had a higher body mass index (31.5 [obese] versus 28.1 kg/m^2 [overweight]), a higher waist/hip ratio (0.898 versus 0.863), higher fasting plasma glucose (95 versus 85 mg/dl) and fasting insulin (23 versus 15 μIU/ml) concentrations (Haffner *et al.* 1995). Metabolic syndrome is more common among Mexican Americans (Table 1) (Churilla *et al.* 2007; Razzouk and Muntner 2009), and a population with a higher prevalence of metabolic syndrome has a greater risk of T2DM and CVD (Kraja *et al.* 2005; Joy *et al.* 2008). In addition, Mexican Americans with T2DM are more likely (i.e., three times) to develop microalbuminuria, a predictor of diabetic nephropathy, than non-Hispanic whites (Haffner *et al.* 1989; Pugh 1996).

Table 1: Prevalence rates for metabolic syndrome. Rates for metabolic syndrome are more common among Mexican Americans (Cossrow and Falkner 2004).

	Women	Men
Non-Hispanic white	22.8	24.8
African-American	25.7	16.4
Mexican American	35.6	28.3
Other	19.9	20.9

From NHANES 1999–2000 data.

The US is the world leader in the prevalence of obesity and overweight (Francischetti and Genelhu 2007). The health care costs for obesity/overweight in the US are estimated at ~$100 billion each year (Harper 2006), and will reach $1 trillion by the end of 2030 (Wang *et al.* 2008). In addition to these health problems in adults,

childhood overweight/obesity, along with T2DM and metabolic syndrome, have been increasing significantly in the US over the past two decades. It is known to be correlated with a large number of chronic health conditions and illnesses in adulthood (e.g., T2DM, metabolic syndrome, and hypertension). Moreover, about 45 (~ 30%; WHO) million of the world's school-age children are estimated to be obese (Mehta 2007). In the US, between the years 1980 and 2000, the incidence of overweight in children aged 6-11 years more than doubled, while it tripled in adolescents aged 12-19 years (Harper 2006). According to the 2003-2004 National Health and Nutrition Examination Survey (NHANES), the occurrence of overweight in children and adolescents aged 2 to 19 years was 17.1% (Ogden *et al.* 2006).

Epidemiology of MS in children

Obese/overweight Mexican American children are at greater risk with 20% higher prevalence for the metabolic syndrome (Butte *et al.* 2005) (estimates vary from 5.6% - Cook *et al.* 2003; and 12.9% - de Ferranti *et al.* 2004), and is still higher (30%) if there is a family history of T2DM (Cruz *et al.* 2004). Among Mexican American children who are diagnosed with T2DM, 90% are found to be obese and 75% have two or more abnormal lipid levels (high total cholesterol [TC] and/or high low-density lipoprotein [LDL] cholesterol and/or high triglycerides [TG]) (Fortmeier-Saucier *et al.* 2008). These findings suggested as the body mass index increased (i.e., overweight/obese), the probability of abnormal total cholesterol, triglycerides and LDL levels also increased. Previous studies showed a correlation between body mass index and lipid levels (Lamon-Fava *et al.* 1996). In another study (Movassaghi *et al.*

2007), the children of parents with metabolic syndrome had higher serum triglycerides, LDL, total cholesterol, fasting blood glucose, and lower HDL, indicating a familial aggregation of metabolic syndrome. In Mexican Americans, metabolic syndrome rates are high and correlated with age (Smith *et al.* 2005). MS is greater in Mexican Americans, especially women (age effects and sex dimorphism are evident), and in children ethnic differences are observed in the expression of MS at a young age (Cossrow and Falkner 2004).

A number of studies have been conducted to verify the factors underlying the metabolic syndrome structure (e.g., Arya *et al.* 2002b). Such studies have found more than one factor responsible and interaction of separate biological processes for the underlying structure of metabolic syndrome-related multivariate data. Therefore, it is likely that the association between obesity (central obesity in particular) and insulin resistance (along with hyperinsulinemia) could be the instigating factor in the development of metabolic syndrome (Arya *et al.* 2002b; Caterson and Gill 2002; Srinivasan *et al.* 2002; Fezeu *et al.* 2007). The underlying biological basis for ethnic difference in the predisposition to diabetes could be attributable to insulin resistance [as measured by Homeostatic model assessment of insulin resistance [HOMA-IR], an indirect measure of insulin resistance] (Huang *et al.* 2002). To some extent the higher rates of obesity-related diabetes in Mexican American population are explained by insulin resistance which is a strong correlate of obesity. In addition, the offspring of affected parents are more vulnerable and exhibit higher risk to IRS or MS.

Using NCEP/ATPIII definition for metabolic syndrome and NHANESIII data including 8814 men and women aged ≥20 years, the age adjusted prevalence of metabolic syndrome was found to be 24.0% in men and 23.4% in women representing a cross-sectional sample of US population (Ford *et al.* 2002). The prevalence showed age-dependence and also differed among ethnic groups. The highest prevalence was among Mexican Americans (31.9%) and the lowest among non-Hispanic whites (23.8%), African Americans (21.6%) and other ethnic groups combined (20.3%) (Ford *et al.* 2002). Using NCEP/ATPIII definition for metabolic syndrome and NHANESIII data including 2430 children aged 12-19 years, and the prevalence of metabolic syndrome was found to be 4.2% (6.1% in males and 2.1% in females) with the highest in Mexican Americans (5.6%), whites (4.8%), and then African Americans (2.0%). Also, 28.7% of overweight adolescents met the condition of metabolic syndrome even after stratified by body mass index (Cook *et al.* 2003). Therefore, these rates suggest that ~1million adolescents are affected in the US, and 47 million adults are estimated to be affected with metabolic syndrome in the US (Cook *et al.* 2003). In a study based on 126 overweight Hispanic children aged 8-13 years, the prevalence of metabolic syndrome was found to be 30% (Cruz *et al.* 2004). This study also indicated an association between insulin resistance and the components of the metabolic syndrome along with a family history of T2DM and overweight.

Blood pressure and its metabolic correlates

Hypertension can be classified into two types: essential or primary hypertension – chronically elevated blood pressure occurring in the absence of other predisposing conditions, and secondary hypertension – elevated blood pressure mainly due to kidney diseases. This latter condition depends upon regulation of blood pressure and which in turn depends on blood transport system and plasma volume. Plasma level, in relation to proteins and blood cells, relies on sodium (NA+) and potassium (K+) ion concentrations in the blood. This sodium and fluid balance is regulated by renin-angiotensin-aldosterone system (RAAS) in the blood. The RAAS pathway and regulation of blood pressure is illustrated in Figure 4. When the body is deficient in fluids, then the pituitary gland releases antidiuretic hormone (ADH) to force the kidneys to reduce water filtration. This reduced fluid volume eventually leads to a fall in blood pressure that is sensed by receptors on the kidneys and induces the release of rennin enzyme. Renin, coupled with angiotensin (which is released by liver) forms angiotensin I, which is then converted into angiotensin II catalyzed by angiotensin converting enzyme (ACE). This happens during low sodium and potassium concentrations. RAAS stimulates absorption of sodium and also aldosterone production by the adrenal glands. With the help of these enzymes, RAAS influences hemodynamics and vascular tone by maintaining the water and sodium balance. Therefore, any changes in the RAAS pathway such as variation in genes encoding renin, angiotensinogen, ACE, & angiotensin receptors may lead to essential hypertension (Cooper *et al.* 1999; Lely 2007).

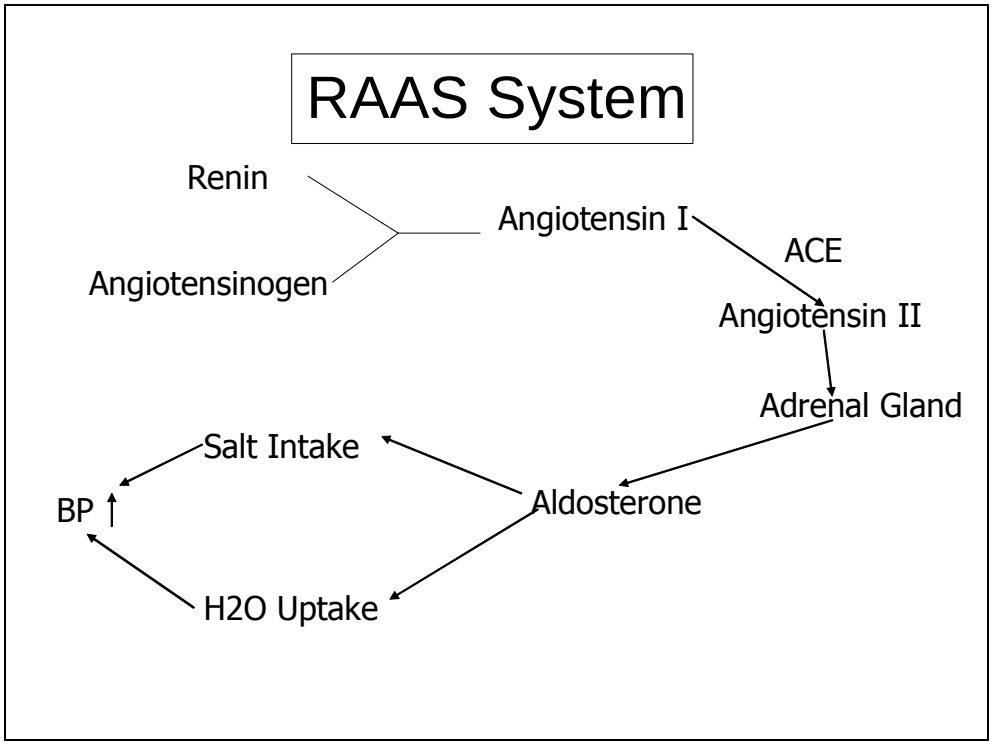

Figure 4: The Renin-Angiotensin-Aldosterone System (RAAS).

Since the late 1980s, the prevalence of elevated blood pressure has been increasing among US children and adolescents. Similar increase is observed for general forms of obesity, in particular abdominal obesity. Both obesity and hypertension are significantly associated (Cossrow and Falkner 2004). The rise in hypertension can be attributed to obesity to some extent (Figure 5) and also clustering of risk factors due to metabolic syndrome in children (Din-Dzietham *et al.* 2007). Besides, obese children are at higher risk (~3 fold) for hypertension than nonobese children (Sorof and Daniels 2002). A high prevalence of overweight is observed in Mexican American adolescents. For most ages, males had a higher prevalence of

overweight than females. In overweight Mexican American adolescents, hypertension establishes early; and, higher systolic blood pressure levels are observed after adjusting for other risk factors (Forrest and Leeds 2007). Once hypertension in children was considered as secondary and rare, but the hypertension in children seems primary and has become common and increasingly associated with obesity and other risk factors including a family history and ethnic predisposition to hypertension and related diseases. Obesity-related hypertension appears to be a cumulative effect of insulin resistance, abnormalities in vascular structure and function and several other associated factors (Sorof and Daniels 2002; Sorof *et al.* 2004). Elevated blood pressure at a young age is associated with elevated levels of blood pressure and overweight later in life (Colin-Ramirez *et al.* 2009).

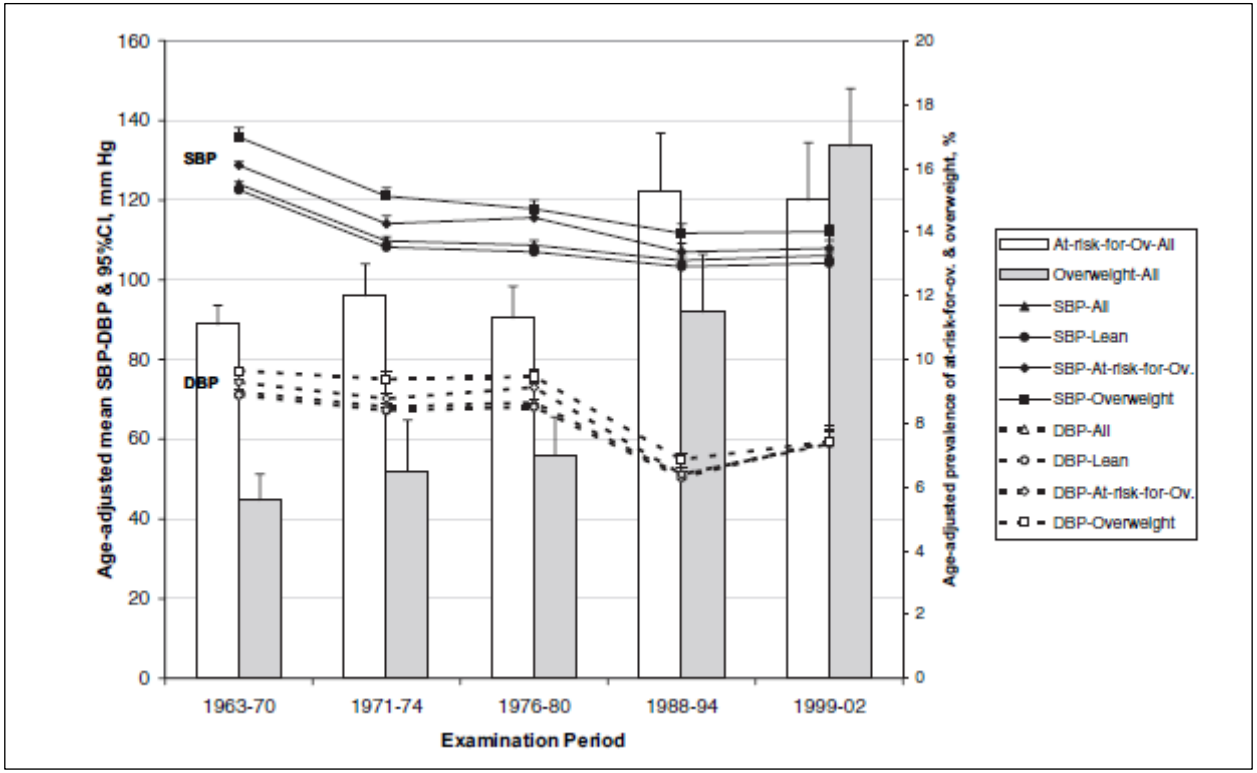

Figure 5: Showing systolic blood pressure and diastolic blood pressure levels over time in overweight and at risk for overweight youth (Din-Dzietham *et al.* 2007).

In Mexican school children aged 8 to 10 years, elevated blood pressure is associated with overweight in both girls and boys (Genovesi *et al.* 2008) and also a predictor for elevated blood pressure levels (SBP and/or DBP ≥95% for gender, age and height) in adulthood (Ostchega *et al.* 2009; Colin-Ramirez *et al.* 2009). Based on the NHANES cross-sectional data including Mexican Americans, non-Hispanic whites and non-Hispanic blacks from three time periods: 1) NHANESIII (1998-1994) sample of which 4,673 children were interviewed and examined aged 8-17 years; 2) NHANES (1999-2002) sample of which 5,110 children were interviewed and examined aged 8-17 years; 3) NHANES (2003-2006) sample of which 4,662 children were interviewed and examined aged 8-17 years, obese children and adolescents were found to have significantly higher levels of pre-elevated (SBP and/or DBP ≥90 but <95% for gender, age and height) and elevated blood pressure values (Ostchega *et al.* 2009). There is a significant increase in pre-elevated blood pressure over time in Mexican American girls aged 8-12 years, and for non-Hispanic black adolescent girls aged 13-17 years. Also, there is a significant association of elevated blood pressure over time for adolescent girls aged 13-17 years (Ostchega *et al.* 2009). The detection and control of hypertension is low in Mexican Americans, which is as well an added concern. Likewise, the hypertension control differs by ethnicity even after accounting for patient characteristics, treatment, and adherence to treatment. The likelihood of uncontrolled hypertension is higher in Mexican Americans, despite medication adherence, salt restricted diets (Natarajan *et al.* 2005).

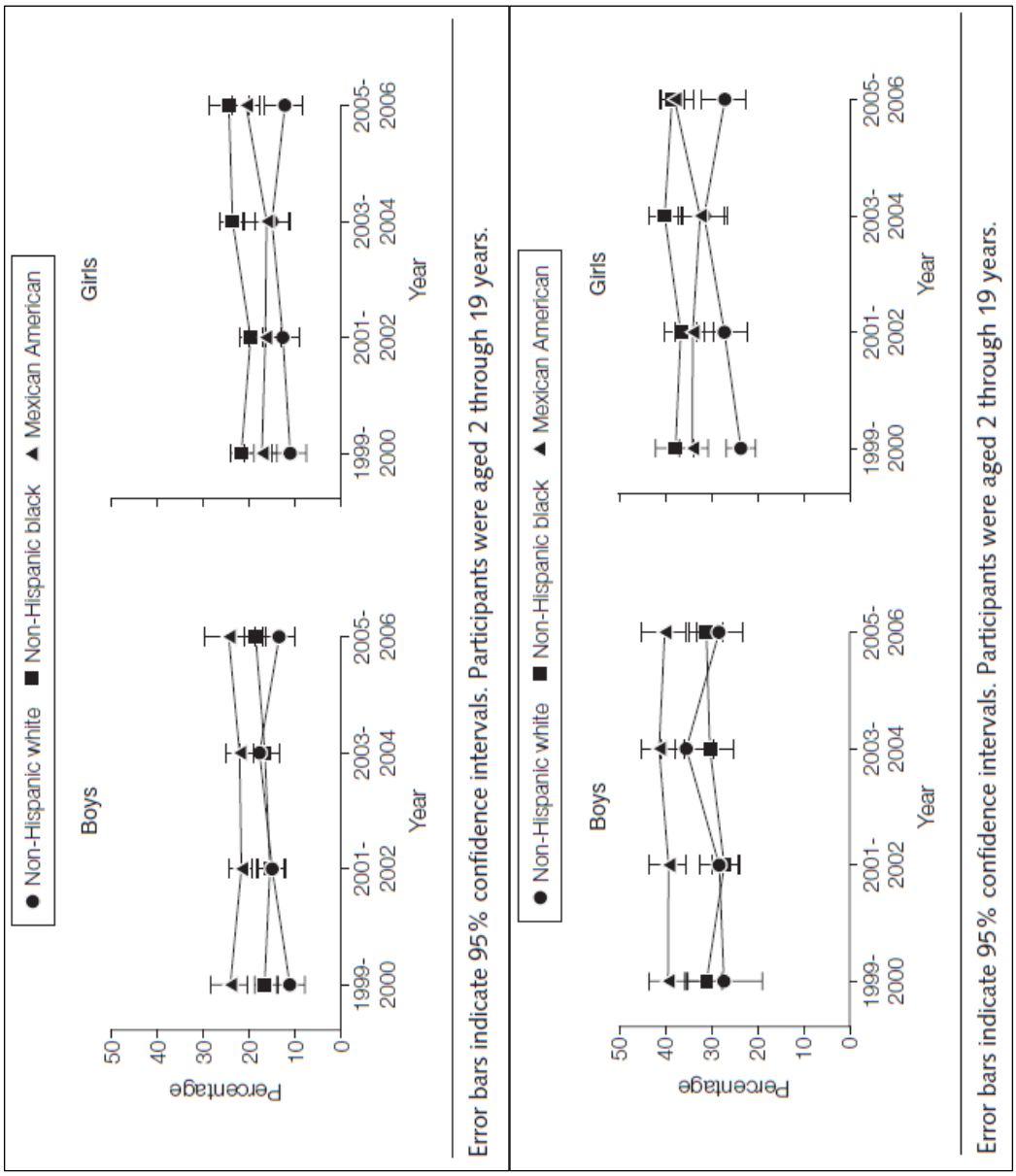

Figure 6: BMI for age at or above 95th percentile (left) and 85th percentile (right) by ethnicity during 1999-2006 in non-Hispanic whites, non-Hispanic blacks, and Mexican Americans (Ogden *et al.* 2008).

Mexican American boys are more likely to have a high body mass index at all ages compared to non-Hispanic whites or blacks. Mexican American girls have a similar high body mass index to non-Hispanic blacks and a slightly higher risk than non-Hispanic whites at most ages represented in Figure 6 (Ogden *et al.* 2008). High body mass index is associated with elevated blood pressure levels in both children and adults (Ostchega *et al.* 2009). Children with BMI $\geq 95^{th}$ percentile (obese) have higher prevalence of hypertension than children in other percentile categories (Sorof and Daniels 2002; refer Figure 7). Several studies showed a significant positive association among body mass index, waist circumference and systolic blood pressure, but not with diastolic blood pressure. The association among body mass index, waist circumference and diastolic blood pressure is unclear may be due to the fact that diastolic blood pressure was not analyzed independently from systolic blood pressure (Colin-Ramirez *et al.* 2009). Mexican American children in the mixed hypertensive group (both systolic and diastolic blood pressure) have higher weight, body mass index and waist circumference values in parallel with higher prevalence of overweight and obesity including elevated systolic and diastolic blood pressure levels (Colin-Ramirez *et al.* 2009).

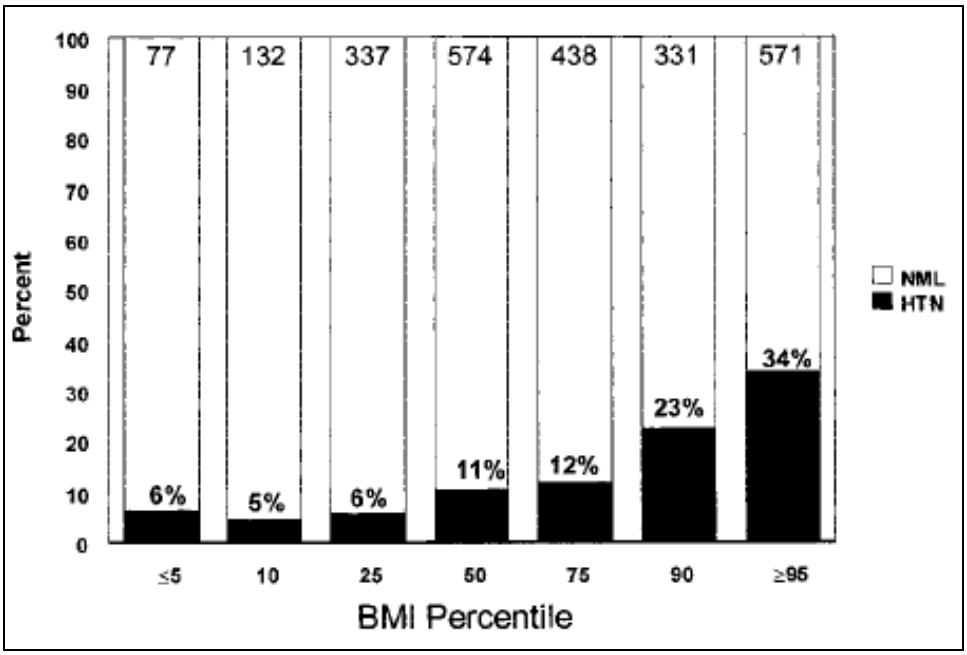

Figure 7: The distribution of hypertension within each BMI percentile category in normotensives and hypertensives (Sorof and Daniels 2002).

Both body mass index and waist circumference (a measure of abdominal obesity) are associated with elevated levels of systolic and diastolic blood pressure levels (given in Figure 8). However, waist circumference is a good predictor of blood pressure even after correcting for body mass index (Genovesi *et al.* 2008). Also, increase in adiposity over time including increased body mass index, waist circumference, skin-fold thickness is associated with increased blood pressure (systolic) levels (Jafar 2009). Whereas, total fat intake showed positive association with diastolic blood pressure (Colin-Ramirez *et al.* 2009). These increasing rates of

obesity and its co-morbid disease conditions are of major concern to the general public, health officials and policy makers because of their implications on Americans' health (AHA 2005).

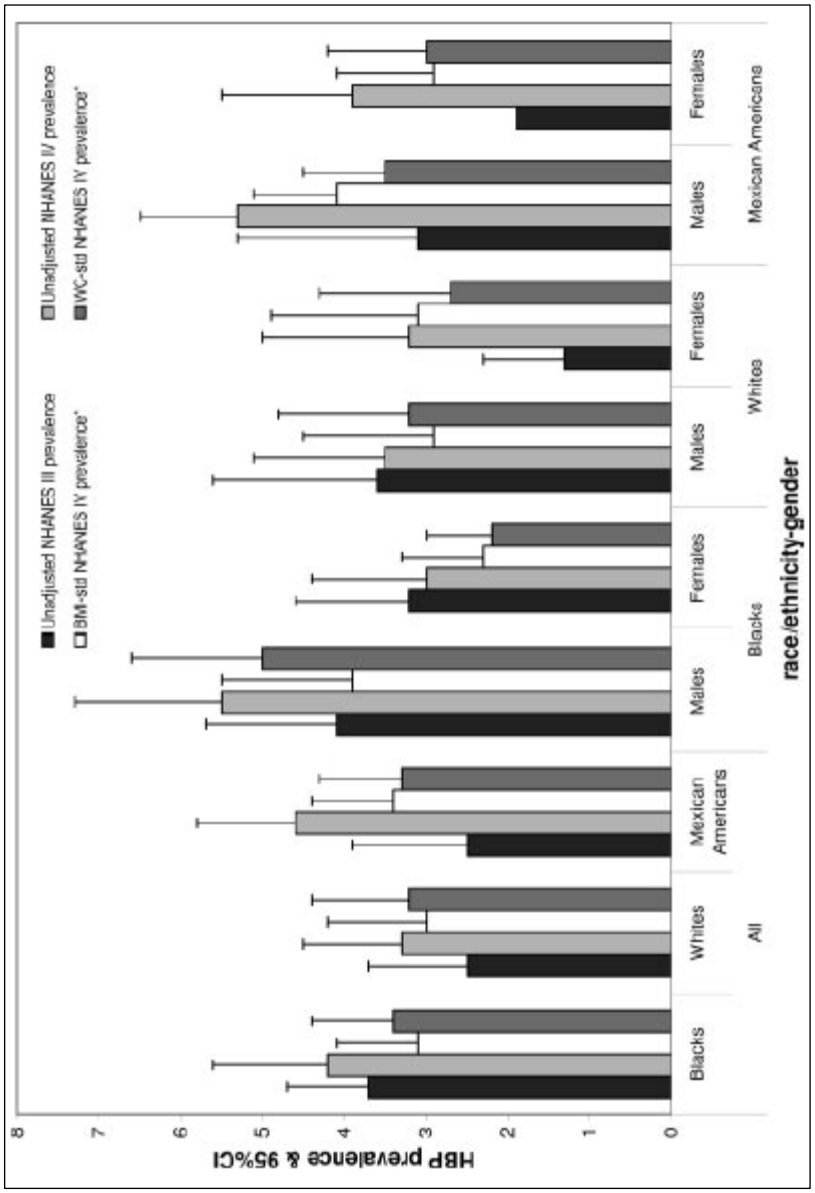

Figure 8: Rise in obesity [black bars] and hypertension [light gray bars] along with BMI [white bars] and waist circumference [dark gray bars] in Blacks, whites, and Mexican Americans (Din-Dzietham *et al.* 2007).

Multifactorial origins and interactions of MS-related phenotypes

The complex phenotypes of obesity, T2DM, and hypertension are influenced by genetic and environmental factors and their interactions. Aside from genetic influences, the rapid rise in the prevalence rates of obesity and T2DM in recent decades is attributable to changing environmental factors including socioeconomic and life style factors (e.g., poor dietary habits [high calorie intake] and reduced physical activity levels) and obesogenic environments such as fast food restaurants and increased television viewing time leading to positive energy balance and resulting in an accumulation of body fat (Moore 2000; Harper 2006; Mehta 2007; Gable *et al.* 2007). Conversely, increased physical activity and fitness could lead to favorable lipid profiles and improvement in insulin sensitivity (Isomaa 2003; CDC 2004). Another potential contributing factor to the development of T2DM or insulin resistance in children is puberty (ADA 2000; Goran *et al.* 2003). However, there are certain intervention approaches (including lifestyle modifications) that will help a child from gaining weight or reach overweight category (Golan 2006; Williamson *et al.* 2008; Swinburn 2009). Not all children respond in a similar way, and there is great inter-individual variation to these interventions indicating a possible influence of genes and their interactions with lifestyle adjustments. The response variations in children for these short-term lifestyle interventions can be attributed to genetic influences or some specific variants in disease susceptibility genes and to gene-by-lifestyle (G x LS) interaction influences (Shiwaku *et al.* 2003; de Luis *et al.* 2006; Mitchell *et al.* 2008; Moore *et al.* 2008).

It is well established that obesity and T2DM phenotypes have strong genetic determinants (e.g., Duggirala *et al.* 1999; Comuzzie 2002). The major advances in molecular genetic and statistical genetic techniques and the availability of the human genome data helped the genome-wide searches to locate disease susceptibility genes. Techniques include linkage, association, and admixture mapping (Duggirala *et al.* 1999, 2000a, 2001; Comuzzie 2002; Arya *et al.* 2002a, 2004; Zhu *et al.* 2005; Tian *et al.* 2006; Grant *et al.* 2006; Frayling 2007). Importantly, in very recent years, the genome-wide association studies have greatly enhanced our understanding of the genetic architecture of obesity and T2DM. Variants associated with susceptibility to T2DM are the Pro 12 Ala substitution, the Ala allele was common (85%) and discovered by testing candidate genes with a relative risk of ~0.8 (Altshuler *et al.* 2000); and cysteine protease gene calpain 10 (CAPN10) (Horikawa *et al.* 2000) discovered in Mexican Americans with the help of two intronic SNPs and an indel marker the susceptibility of this genotype is associated with diabetes. The genes of these complex traits can be mapped by different approaches.

Methodological approaches to map genes of complex traits

The two general strategies used to map genes for complex traits are genome-wide linkage analysis and genome-wide association study approach. Novel integrative approaches for the identification of candidate genes in hypertension combine linkage and gene expression profiling, and the dissection of physiological phenotypes (Hubner *et al.* 2005). Using the genome-wide linkage analysis, Duggirala *et al.* (1999) identified

a T2DM susceptibility locus on chromosome 10q in Mexican Americans. Also, there is evidence for major loci influencing blood pressure and lipoprotein metabolism (Krushkal *et al.* 1999; Duggirala *et al.* 2000b; Arya *et al.* 2002b; Rice *et al.* 2002).

So far many genome-wide linkage studies, gene candidate analysis, whole genome-wide association studies of hypertension and blood pressure-related phenotypes have been published (OMIM 145500; Mocci *et al.* 2009). However, no single genomic region has a large effect on hypertension (Mocci *et al.* 2009), but several genomic regions likely to contain essential hypertension-susceptibility loci: 1q, 2p, 2q, 3p, 6q, 11q, 12q, 13 q, 15q, 18q, and 19p [chromosome number; p - short arm of the chromosome; q - long arm of the chromosome] (Rice *et al.* 2000; Mocci *et al.* 2009). Chang *et al.* (2007) characterized linkage region 1q23-q32, validated by several other studies and supported by blood pressure -related quantitative trait linkage mapping studies. The results indicated that 1q harbors multiple essential hypertension-susceptibility genes that affect blood pressure levels. Variance components linkage analysis applied to 1,010 Euro-Americans and 816 Afro-American samples of blood pressure found strongest evidence for linkage with a maximum logarithm of odds (MLOD) score of 3.2 on chromosome 1q. This finding is also verified by other studies including 16 additional STRs genotyped in the region with 2 peaks: 4.3 and 1.8 MLOD. Out of the nine positional candidate genes located in close proximity to the MLOD peaks, three were potentially related to blood pressure regulation (Xiao *et al.* 2009): (1) ATP1B1 - a transporter of Na+ and K+ across plasma membrane, regulates Na+ absorption, vascular smooth muscle tone regulation, decreased Na, K-ATPase activity

precedes development of hypertension in animal models; (2) RGSs (regulator of G-protein signaling) - mediates vasoconstriction through angiotensin, plays role in angiogenesis, may sense hemodynamic change and remodeling of arteries ; (3) SELE (encodes E-selectin, an endotheial specific adhesion molecule and marker of endothelial function; activated endothelium promotes formation of atheroma, reduces elastic properties of arterial wall and alters responsiveness to vasoactive stimuli - part of EH Endothelial dysfunction or impaired vasodilation results in elevated plasma levels of soluble selectin - common feature of essential hypertension). These findings establish a genetic association between blood pressure regulation and response to stress (Henry 2003; Isomaa 2003; Goldstein 2003; Tracy 2003; Cordain *et al.* 2003; Malnick *et al.* 2003; Rowley *et al.* 2003; Chang *et al.* 2007; Xiao *et al.* 2009).

A high prevalence (≥70%) of obesity-associated hypertension in French Canadian families and a genome-wide scan using multipoint linkage analysis found significant loci on chromosome 1 (D1S1597) and 11 (D11S1999) (Pausova *et al.* 2005). Genome-wide scans using factor analysis found a quantitative trait locus on chromosome 15q15 for blood pressure in whites (Kraja *et al.* 2005); quantitative trait locus for systolic blood pressure on 20p12 in Pacific Islanders of Kosrae (Shmulewitz *et al.* 2006). In addition, very recently, some studies have used a genome-wide association study (GWAS) approach to localize susceptibility genes for hypertension or blood pressure (e.g., Levy *et al*. 2009; Adeyemo *et al*. 2009). Several novel susceptibility genes/variants for T2DM/obesity (e.g., transcription factor 7-like 2 [*TCF7L2*] reported in Mexican Americans (Almasy and Blangero 2009; McCarthy *et*

al. 2008; Prokopenko *et al.* 2008) and fat mass and obesity associated [*FTO*] genes) have been identified in recent years, many with subsequent replication (reviewed by Perry and Frayling 2008, Dina 2008). Montasser *et al.* (2009) used both genome-wide linkage and candidate gene association approaches and detected a major quantitative trait locus on chromosome 15q for systolic blood pressure in nonsmokers indicates the presence of a locus that influence blood pressure via G x S interactions. Presently, several research groups are engaged in positional cloning efforts to identify the disease-specific functional variants.

Despite the above advances in hypertension susceptibility gene discovery using data mainly from adult populations, as discussed previously, little is known about the factors that underlie the phenotypic expression of components of MS including blood pressure as well as the clustering pattern of these components in children and adolescents, especially in Mexican Americans who are at high risk for the development of T2DM, obesity, hypertension and MS. Therefore, the major purpose of this dissertation is to address the issues of blood pressure and its relation with other components of MS in children, using data from a well-established cohort of Mexican American children. The next chapter describes the genetic epidemiological techniques that have been implemented for examining the genetic architecture of blood pressure and other components of MS.

CHAPTER THREE: MATERIALS AND METHODS

SAFARI Study Design and Sample

SAFARI (**S**an **A**ntonio **F**amily **A**ssessment of Metabolic **R**isk **I**ndicators in Youth) project is a multidisciplinary study of genetic and environmental influences and their interactions on various metabolic syndrome related traits in children. The study is also designed to explore the extent of similarities between adults and children regarding the precursors of metabolic syndrome, and it seeks to verify whether metabolic syndrome in children has distinct etiology. SAFARI children, aged 6-17 years, are offspring of the adult family members who participated in three ongoing large Mexican American family studies in San Antonio, Texas (Figures 9 and 10): the San Antonio Family Heart Study (SAFHS, PI: Dr. John Blangero), the San Antonio Family Diabetes/Gallbladder Study (SAFDGS, PIs: Dr. Donna M. Lehman and Dr. Ravindranath Duggirala), and the Veterans Administration Genetic Epidemiology Study (VAGES, PI: Dr. Ralph A. DeFronzo). The SAFARI study is an ongoing 5-year long project (i.e., 03/01/2005 – 02/28/2010) funded by NICHD/NIH (R01 HD049051, titled: The Metabolic Syndrome in Mexican American Children, PI: Dr. Ravindranath Duggirala). The conduct of SAFARI study has been approved by the Institutional Review Board (IRB) at the University of Texas Health Science Center at San Antonio (UTHSCSA), San Antonio, Texas.

The recruitment of SAFARI children began on September 21, 2005, and ended on February 27, 2009. A total of 607 Mexican American children were enrolled in SAFARI study. Metabolic syndrome-related data have been collected

from each child. After excluding three children that were found to have T2DM at SAFARI clinic examinations, this study will examine data from 604 nondiabetic children and adolescents. Of the children in SAFARI, almost 80% live in the southern and western part of Bexar County. Most of the participants from this study were from low-income neighborhoods including Barrio region in downtown, suburban regions and transition regions between the two in San Antonio. The number of participants by Zip Code in San Antonio, Bexar County, Texas is shown in Figure 10 (i.e., distribution of only 544 children is shown). The San Antonio Metropolitan Health District identified 10 Zip Codes (78201, 78207, 78210, 78211, 78221, 78223, 78227, 78228, 78237 and 78242) as poor and high risk areas for ongoing public health problems in 2006 (http://www.sanantonio.gov/heath). The average median household income of these 10 Zip Codes was $26,824 (http://factfinder.census.gov). These 10 Zip Code areas represent about 30% of the Bexar County population including 82% of Hispanics by ethnicity (http://factfinder.census.gov). About 50% of SAFARI children were from these Zip Code areas represented in red color (refer Figure 10).

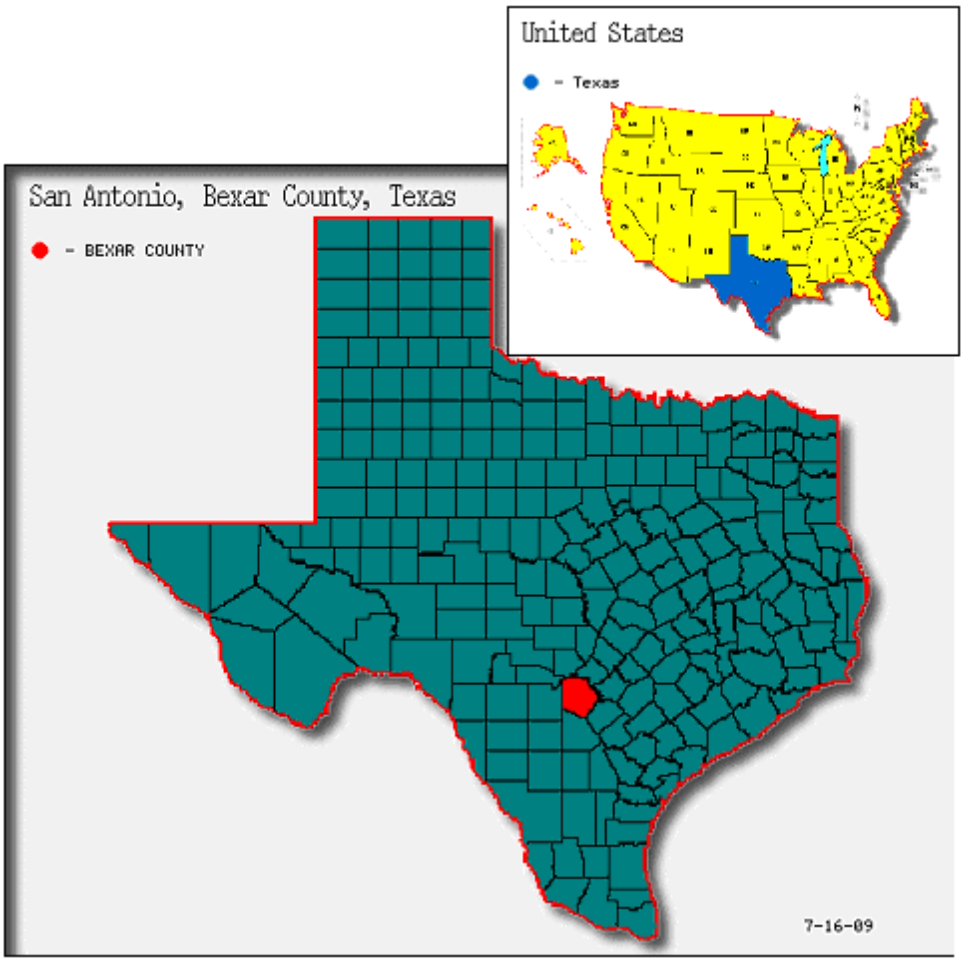

Figure 9: Map of Texas showing Bexar County and San Antonio (map retrieved on 7/16/09 using http://monarch.tamu.edu/~maps2/diy_map.htm#bottom).

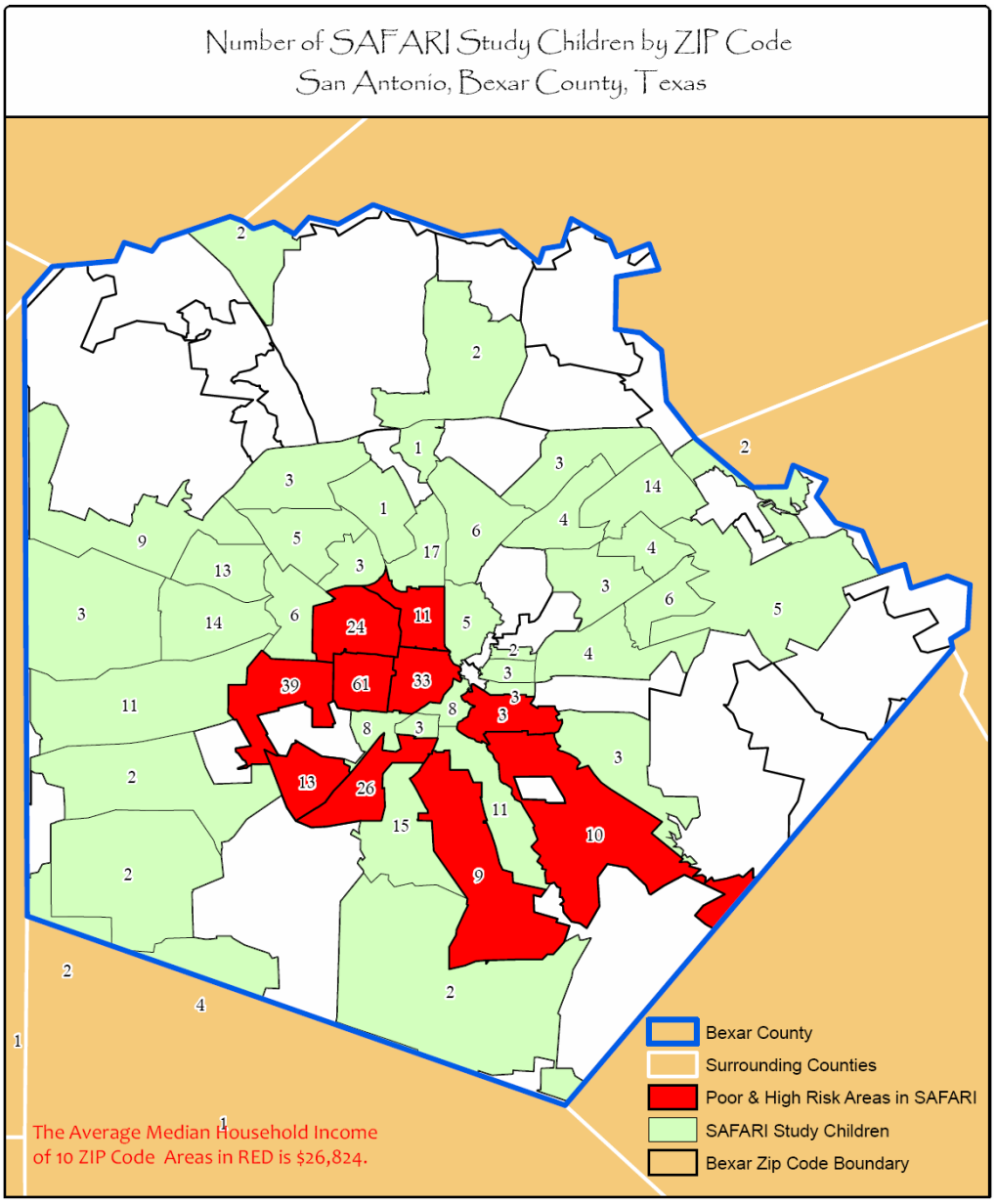

Figure 10: Showing the low-income neighborhoods in red and number of SAFARI study children by Zip Code in San Antonio, Bexar County, Texas (Image courtesy: Duggirala).

San Antonio city is the seat of Bexar County (http://www.naco.org) and is the second largest city in the state of Texas (Figure 9). Texas is the second largest state in the US in both area and population. Over 1/3rd (35.5%, most are Mexican Americans) of Texas residents are of Hispanic origin (http://factfinder.census.gov). Although a majority of Hispanics are recent immigrants from Mexico, some Tejano ancestors have multigenerational bonds to 18th century Texas. Mexican Americans are a major part of the largest minority group-Hispanics in the US. Accordingly ~44 million (14.7%) in the US are Hispanic in origin; in San Antonio, ~61% of the total population are Hispanic or Latino in origin. San Antonio has the largest Spanish settlement in Texas (http://factfinder.census.gov). According to the 2000 census data, 42% of the total population in San Antonio comprises of Mexican Americans (http://factfinder.census.gov). These Mexican Americans represent an admixed population, with admixture proportions approximately 64% from Native Americans and 36% from Europeans (Bertoni *et al.* 2003).

The SAFARI children and adolescents, aged 6-17 years, represent 368 nuclear families as shown in Table 2, and the average number of children per nuclear family is ~2 (range 1-5 children). Given that these nuclear families are extensions of the original SAFDGS, SAFHS, and VAGES families, the SAFARI children represent the bottom generations of these families (refer Figure 11 for an example pedigree). The 604 children have generated a total of 2,847 relative pairs including 317 sibling pairs, 491 first-cousin pairs, 213 first-cousins, once removed, 523 second-cousin pairs, and, 369 third-cousin pairs and others contributed to the present genetic analyses listed in

Table 3 along with the relationship coefficients which refers to two times the coefficient of kinship of two individuals (Coletta *et al.* 2009).

Table 2: The distribution of SAFARI nuclear families by study.

Study	**No. of nuclear families**
SAFHS	185
SAFDGS	72
VAGES	111
Total	**368**

Table 3: The distribution of Relative Pairs in SAFARI children.

Relative pair	**Relationship Coefficient***	**Number of Pairs**
Siblings	0.50000	317
Half siblings	0.25000	89
First cousins	0.12500	491
First cousins (once removed)	0.06250	213
Half first cousins	0.06250	59
Second cousins	0.03125	523
Second cousins (once removed)	0.01562	370
Half second cousins	0.01562	170
Third cousins	0.00781	369
Third cousins (once removed)	0.00391	104
Fourth cousins	0.00195	23
Others	0.25 – 0.00003	119
Total pairs	**-**	**2,847**

* The relationship coefficient refers to two times the coefficient of kinship of two individuals.

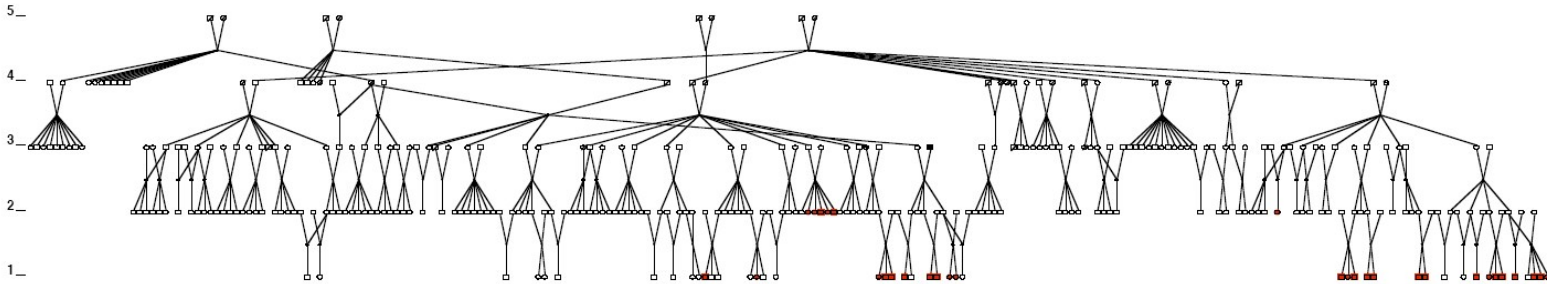

Figure 11: An example pedigree showing SAFARI children in red i.e., bottom generations (Image Courtesy: Duggirala).

Informed consent (assent in some cases) was obtained from each SAFARI participant's parent(s)/participant to be enrolled in the SAFARI study, following the guidelines as approved by the IRB, UTHSCSA, San Antonio. A variety of phenotypic data have been collected from each child on or two visits/days or a single visit/day, using standardized methods given in Table 4. Hence, children had to come twice or once with their parents or guardians to complete the clinical examinations and questionnaires. All enrollment activities together with the clinical examinations were held at the Texas Diabetic Institute (TDI). All clinical examinations were done under the supervision of Drs. Daniel Hale (Medical Director of SAFARI), Jane Lynch and Rolando Lozano, who are well-known pediatric endocrinologists. Ms. Sharon (SAFARI study coordinator), Dr. Richard Granato and Mr. Roy Resendez were involved in the recruitment activities of SAFARI. All of them have excellent rapport with both parents and children.

The required phenotypic data were collected with the help of trained nurses (Ms. Margaret Fragoso and Ms. Rhonda Lyons [to minimize the interobserver error]) under the supervision of pediatric endocrinologists (Drs. Hale, Lynch and Lozano). The pedigrees were updated with the newly collected information by Ms. Fowler and Dr. Granato. Blood samples were collected by the LVN-phlebotomist (LVN – Law Enforcement Medical Services) after a 12-hour fast and 2-hours after glucose load for assessment of various MS-related phenotypes. About 40ml of blood (including the 4 oral glucose tolerance test (OGTT) samples of 5 ml each) was drawn from each child to carry out various laboratory tests as well as to extract DNA. To assess renal

function impairment, morning urine samples were collected. Types of tubes to be drawn were determined by the phenotype assay specific protocols. The LVN/Nurse Coordinator Ms. Fragoso was responsible for the performance of OGTT on the children recruited for this study. The LVN-phlebotomist Ms. Lyons was responsible for the processing and distribution of samples. All fasting plasma glucose and OGTT analyses, using the glucose-oxidase method on a Beckman Glucose Analyzer 2 (Beckman, Fullerton), were carried out at the TDI, whereas other blood samples were analyzed at the Population Genetics Phenotyping Laboratory, Department of Genetics, SFBR, directed by Dr. John Blangero and co-directed by Dr. Joanne Curran.

MS-related phenotypes to establish the precursors of metabolic syndrome in children and adolescents

Data Collection

To establish the precursors of metabolic syndrome, various phenotypes were measured including obesity, impaired fasting glucose, impaired glucose tolerance, insulin resistance, dyslipidemia, hypertension, and microalbuminuria. The information on environment-oriented measures including physical activity and fitness, pubertal status, dietary intake, and television-viewing time were collected using questionnaires. The collected phenotypic data and components of the clinical examination are outlined in Table 4.

Table 4: Phenotypic data collected over two clinical visits are listed (Courtesy: Duggirala).

Visit One	
•	Completion of consent process
•	Anthropometry including height, weight, waist circumference, and body composition estimated by bioimpedence
•	Blood pressure measurements
•	Fasting blood work for glucose, insulin and lipid profile including total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides. OGTT with sample collection at six time points (i.e., -15, 0, 30, 60, 90 and 120 minutes after 75-gram glucose load including two baseline [i.e., fasting at -15 and 0] and four OGTT for glucose and insulin measurements)
•	Urine sample collection (total protein, creatinine and albumin)
•	Resting energy expenditure (REE) measurement
Visit Two	
•	DEXA (fat mass, percent body fat, lean body mass)
•	Harvard Step Test (a measure of physical fitness)
•	Medical history questionnaire
•	Dietary intake history
•	Television-viewing and leisure-time questionnaire
•	Physical activity questionnaire

HDL high-density lipoprotein cholesterol, *LDL* low-density lipoprotein cholesterol, *OGTT* oral glucose tolerance test, *DEXA* dual energy X-ray absorptiometry.

Anthropometry and body composition

Using standard procedures, height was measured using wall-mounted height rod (Ayrton Stadiometer, model S100) and weight was measured with a digital scale (Scale-Tronix, Inc. Wheaton, IL) and the highest value recorded. BMI equals weight in kilograms divided by height in meters squared. This continuous variable was dichotomized into two traits i.e., overweight (BMI $\geq 85^{th}$ percentile for age and sex) and obese (BMI $\geq 95^{th}$ percentile for age and sex) for understanding the severity and variability in adiposity measures. Waist circumference was obtained using a flexible

measuring tape. Body composition (fat mass, percent body fat and lean body mass) was analyzed with the use of a dual-energy-X-ray absorptiometry (DEXA scan, QDR 4500W/CE, Hologic, Inc. Bedford, MA).

Blood pressure measures

Systolic (SBP) and diastolic (DBP) values were obtained by the trained nurses, using a Welch Allyn Spot Vital Signs Monitor. Three readings were recorded and the average of the last two considered as a final measure. Again, the blood pressure data were dichotomized in the analyses to understand the severity of hypertension. Blood pressure $\geq 90^{th}$ percentile for sex, age, and height (i.e., SBP or DBP $\geq 90^{th}$ percentile) is considered as elevated blood pressure [EBP] or hypertension (http://www.cdc.gov/nccdphp/dnpao/growthcharts/resources/sas.htm; National High Blood Pressure Education Program Working Group on Hypertension Control in Children and Adolescents 1996)

MS-related phenotypes assayed at SFBR Population Genetics Phenotyping Laboratory

The blood chemistry/metabolic variables were measured at the SFBR lab facilities. Urine albumin excretion (UAE) is an important phenotype related to renal function impairment and diabetic nephropathy, which is measured as urine microalbumin to creatinine ratio [mg/gm] (ACR). ACR was measured in the morning urine samples using microalbumin/creatinine assay kits either by calorimetric or radioimmunoassay method (Siemens, Tarrytown) and analyzed by Bayer DCA 2000 analyzer at the TDI. Microalbuminuria (MA) is defined as ACR of ≥ 0.03 and <0.30,

while macroalbuminuria is defined as ACR of ≥0.3 (not considered for this study). MA is considered for this study as an early maker of renal function impairment.

Fasting blood work

Using the following procedures, the serum lipid panel was assayed in the Biochemical and Molecular Genetics Laboratory at SFBR. Total cholesterol was measured enzymatically using a kit (H/P Reagent from Boehringer Mannheim). HDL cholesterol was measured enzymatically using the same reagent as for total cholesterol, but after the precipitation of LDL cholesterol and VLDL cholesterol with a mixture of dextran sulfate (50,000 MW) and magnesium chloride. Triglycerides were measured enzymatically using a kit from Stanbio Laboratories (Stanbio Liquicolor Triglycerides). LDL-cholesterol levels were estimated, using the Friedewald formula (Friedwald *et al.* 1972). Serum insulin (RIA, MILLIPORE/LINCO) was assayed using the assay type and source mentioned in the parentheses.

Impaired fasting glucose (IFG ≥100 and ≤125 mg/dl) and impaired glucose tolerance which is 2-hour post-OGTT (IGT ≥140 and ≤199 mg/dl), or both are referred to the condition of pre-diabetes, which raises the risk of diabetes. The various measures of insulin resistance can be derived from the fasting glucose, post-OGTT, and insulin concentrations using methods such as homeostasis model assessment of insulin resistance [HOMA-IR or HOMA] (Matthews *et al.* 1985). HOMA-IR is an indirect measure for insulin resistance which is based on fasting insulin and glucose (Huang *et al.* 2002). There is no single proxy measure available that can account for

the variability in insulin resistance. Therefore, the use of available proxy measures such as fasting glucose and fasting insulin could prove appropriate in the assessment of insulin sensitivity (Huang *et al.* 2002). Here, HOMA-IR is measured as follows: HOMA-IR = [fasting insulin (μIU/ml) * fasting glucose (mmol/l)]/22.5 (Huang *et al.* 2002).

Other measures

Physical fitness

The Harvard step test is a simple test of physical fitness, which requires minimal equipment (Keen and Sloan 1958; Consoalzio *et al.* 1963). A modified Harvard step test, as described by Trevino *et al.* (1999, 2004), is used to measure physical fitness in SAFARI children. A child's lower part of the chest was connected to a heart rate monitor (Polar Vantage XL, Polar Electric Co.) with a transmitter and his/her wrist was connected to a receiver monitor. A baseline heart rate was recorded. Following this, a child was asked to step up and down (both feet) from a stool 30 cm high, 42 cm wide, and 38 cm deep at the rate of thirty cycles per minute. This activity was continued for 5 minutes or until exhaustion. Immediately after the child either completely finished the exercise or stopped the exercise prematurely, heart rates were recorded at 0, 1, and 2 minutes. The heart rate and duration of test in seconds was applied to the following equation to calculate the physical fitness score (PFS):
PFS = Total time of stepping or duration of exercise [in seconds] x 100)/Sum of three heart rates post exercise.

Physical and sedentary activity

Accelerometer-based actical (Mini-Mitter, Bend, OR) activity monitors had successfully been used to estimate physical activity/energy expenditure. Prior to the clinical examinations, participants were asked with the help of their parents to complete 3 days' physical activity logs, with actical readings. Additionally, all children were asked to fill a questionnaire about physical activity and sedentary activity. The physical activity of was evaluated based on time spent per day/week, type and perceived exercise level. The sedentary activity was based upon the information on amount of television-viewing, video games, and other leisure time activities mentioned. The physical activity and sedentary activity for yesterday and usually were calculated (see Appendix 4) using the Girls health Enrichment Multi-site Studies (GEMS) Activity Questionnaire formula described elsewhere (Treuth *et al.* 2003, 2004).

Puberty

Another important measure considered for this study was puberty which was shown to be a significant contributor to insulin resistance (Goran *et al.* 2003). The pubertal status, after physical examination by a physician using the standard Tanner stages, was divided into three categories: pre-pubertal, pubertal and post-pubertal. During the analyses, while estimating heritabilities with significant covariates, two dummy variables were created to account for the pre-, pubertal and post-pubertal differences.

Analytical Approaches

Univariate genetic analysis

A well-established model of multifactorial inheritance that has been used to partition total phenotypic variation of a given trait (e.g., blood pressure) into genetic and environmental components (i.e., univariate genetic analysis) is the variance components analysis (Falconer and MacKay 1996; Almasy and Blangero, 1998). Heritability (h^2) is defined as the total phenotypic variance in a trait (e.g., blood pressure) that is attributed to genetic factors (Falconer and Mackay 1996). There are two forms of heritabilities: broad- and narrow-sense heritabilities. The heritability in broad-sense is defined as the proportion of the total phenotypic variation (V_P) that is determined by genetic components (V_G), which can be further divided into additive (V_A) and dominance (V_D) components.

$$h^2 = V_G/V_P = (V_A+V_D)/V_P \quad (1)$$

The narrow-sense heritability is defined as the total phenotypic variance (V_P) that is attributed to additive genetic factors (V_A). This heritability measure is used for the present study as it considers the degree of resemblance between relative pairs and also considered to be significant in quantitative genetics.

$$h^2 = V_A/V_P \quad (2)$$

Variance components analysis for quantitative or continuous phenotypes

According to the standard quantitative genetic theory, covariances or correlations among biological relatives can be used to partition the total phenotypic variance of a continuously distributed trait (e.g., blood pressure) into its genetic and environmental components (Duggirala 1995; Falconer and Mackay 1996). The total phenotypic variance of a given trait (e.g., blood pressure) can be partitioned into genetic and nongenetic components using a variance components analytical approach. In a simple model, variances or covariances between relatives as a function of the genetic relationships can be specified, and the proportion of phenotypic variance that is attributed to (additive) genetic effects (i.e., heritability: h^2) can be estimated from the components of variance (Hopper and Mathews 1982; Falconer 1989). For such a simple model, the covariance matrix for a family (Ω) is given by:

$$\Omega = 2\Phi\sigma^2_g + I\sigma^2_e \qquad (3)$$

Where Φ is the kinship matrix; σ^2_g is the genetic variance due to additive genetic effects; I is the identity matrix; and σ^2_e is the variance due to individual-specific environmental effects. To better evaluate the genetic basis of a given phenotype, using maximum likelihood techniques, heritability was estimated along with the phenotypic mean and standard deviation and covariates (sex and age effects, puberty and physical fitness), simultaneously (Duggirala *et al.* 2001).

A likelihood ratio test is used to test whether the heritability of a given phenotype ($h^2 = 0$) was zero. This is conducted by setting the likelihood of a restricted

model in which the parameter h^2 was constrained to a value of zero and compared to that of a general model in which the same parameter was estimated. A test statistic can be yielded by doubling the difference in log-likelihoods of these models, which is asymptotically distributed, as a ½:½ mixture of a χ^2_1 distribution and a point mass at 0 (Self and Liang 1987; Duggirala *et al.* 2001). Using likelihood ratio tests, the null hypotheses of no influence of a given covariate ($\beta_{COVARIATE} = 0$) were tested. A given parameter is compared in each of these tests and involves 1 degree of freedom. A statistically significant test, if $p \leq 0.05$ would be considered evidence of a significant, non-zero estimate for a given parameter (Duggirala *et al.* 2001). The analyses of procedures are incorporated in the program SOLAR [Sequential Oligogenic Linkage Analysis Routines] (Almasy and Blangero 1998).

Variance components analysis for qualitative or dichotomous phenotypes

Although the above variance components method is appropriate to quantitative traits, it can be extended to the dichotomous or discrete (disease = yes or no) traits such as hypertension or metabolic syndrome or its components as defined by the NCEP/ATPIII criteria (Duggirala *et al.* 1997; 1999). It is assumed that an individual belongs to a specific disease category if the liability or underlying genetically determined risk exceeds a certain threshold, T, on a normally distributed liability curve (Puppala *et al.* 2006). The liability is assumed to have an underlying multivariate normal distribution with equal unit variances both in the general population and in relatives of affected individuals. The correlation in liability between pairs of individuals will be estimated using the affected status of unrelated individuals and various categories of

relatives (Duggirala *et al.* 1997; 1999; Puppala *et al.* 2006). For a simple model, the correlation (ρ) in liability between individuals i and j is given by:

$$\rho_{ij} = 2\Phi_{ij} h^2 + I_{ij} e^2 \quad (4)$$

Where Φ_{ij} gives the kinship coefficient for individuals i and j; h^2 is the heritability due to additive genetic effects; I_{ij} gives the coefficient for the random environmental effects for individuals i and j; and $e^2 = 1 - h^2$ (Lin *et al.* 2005; Puppala *et al.* 2006). Since calculation of the likelihood for this multifactorial model requires high dimensional integration, the Mendell/Elston algorithm (1974) will be used for evaluation, approximately. Similar to the quantitative traits, the variance components and covariate effects for discrete traits were estimated in likelihood terms, and hypothesis were tested using likelihood ratio tests (Lin *et al.* 2005; Puppala *et al.* 2006). The computer program SOLAR is implemented for this variance components procedure for discrete traits (Almasy and Blangero 1998).

Multivariate variance components analysis: Determination of common genetic influences (i.e., pleiotropy) on correlated phenotypes

By extending the univariate variance component model to the multivariate situation, the bivariate variance components analysis can be used to partition phenotypic correlation (ρ_p) between a pair of quantitative phenotypes (e.g., SBP and HDL cholesterol) into additive genetic (ρ_g) and environmental (ρ_e) components using information on genetic relationships between relatives and with the maximum likelihood technique (Hopper and Mathews 1982, Lange and Boehnke 1983,

Williams-Blangero and Blangero 1993). An extension of this approach can be employed to conduct the bivariate analysis of quantitative and dichotomous (e.g., SBP and overweight) or two dichotomous traits (e.g., hypertension and microalbuminuria) (Williams *et al.* 1999a, 1999b; Burke *et al.* 2000). The nature of the phenotypic correlation (ρ_P) between a pair of traits is given by:

$$\rho_P = \sqrt{h^2_1 h^2_2}\,\rho_G + \sqrt{e^2_1 e^2_2}\,\rho_E \qquad (5)$$

Where ρ_P is the phenotypic correlation; ρ_G is the additive genetic correlation; ρ_E is the random environmental correlation; h^2_1 is the heritability of trait 1; h^2_2 is the heritability of trait 2; e^2_1 is equal to $1-h^2_1$; e^2_2 is equal to $1-h^2_2$. The significance of the phenotypic, additive genetic, and random environmental correlations were determined, respectively by using likelihood ratio tests (Burke *et al.* 2000; Lopez-Leon *et al.* 2009). For example, to determine whether ρ_G between two traits is significantly different from zero or not, the log-likelihood of a model in which the parameter ρ_G was constrained to equal zero was compared with a model where the same parameter was estimated. A test statistic can be yielded by doubling the difference in log-likelihoods of these models, which is asymptotically distributed as a X^2 statistic (Burke *et al.* 2000; Lopez-Leon *et al.* 2009). The difference in the number of parameters estimated in the two competing models equals the degrees of freedom (Burke *et al.* 2000). The bivariate genetic analysis can incorporate covariate effects

(e.g., age and sex). The procedures of bivariate analysis are incorporated in the computer program SOLAR (Almasy and Blangero1998).

Selection of Covariates

In our variance component analysis, a number of covariates (i.e., intermediate physiological phenotypes or environmental factors) of a given disease phenotype (e.g., SBP or DBP) were included in the model, and they might influence the proportion of the total phenotypic variance associated with polygenic effects. The covariate screening options in the program SOLAR is used to identify important covariates of the MS or its related disease conditions. The covariates included in the initial genetic analyses were sex, age, age^2, age*sex, age^2*sex [interaction], puberty, physical activity and fitness effects. However, only significant covariates were considered for the final models. For a given analysis, to minimize the problem of non-normality, outliers, defined as ± 4 standard deviations from the mean were excluded. For the variables such as fasting insulin, HOMA-IR, triglycerides, BMI and body fat - the distributions were skewed, so they were log-transformed.

CHAPTER FOUR: RESULTS

Descriptive characteristics of the study sample

Demographic, hemodynamic, and renal function-related variables

The demographic, hemodynamic, and renal function characteristics of the total study population and boys and girls separately are presented in Table 5. This table describes the following variables used in this study: demographic (age and gender), hemodynamic (systolic blood pressure, diastolic blood pressure, elevated blood pressure [higher systolic or diastolic blood pressure or both based on 90th percentile for age, sex, and height]), and renal-function related (microalbuminuria) traits. The mean age of the SAFARI participants was 11.6 ± 3.4 years, and 50% of the participants were girls. The mean values (mm Hg) for systolic and diastolic blood pressure were 104.2 ± 9.8 and 63.1 ± 7.0, respectively. The systolic blood pressure (boys - 105.7 ± 10.1, girls - 102.7 ± 9.2) was significantly different between sexes (p = 0.000) and diastolic blood pressure (boys - 62.9 ± 7.0, girls - 63.4 ± 7.0) values were not significantly different between boys and girls (see Figure 12).

High blood pressure

Of the total participants, 10.3% of children had SBP greater than or equal to 90th percentile and 6.8% of them had higher DBP, whereas, the prevalence of elevated BP (EBP) or hypertension was 12.6% in the total study population (i.e., either SBP or DBP greater than or equal to 90th percentile). However, the dichotomized blood pressure (normal vs. hypertensive) variables such as SBP≥90 and EBP≥90 were higher in boys (11.6% and 13.6%) than girls (8.9% and 11.6%). In

contrast, DBP≥90 was slightly higher in girls (7.3%) than boys (6.3%) (see Figure 15).

Microalbuminuria

ACR is an important early marker for kidney disease and generalized endothelial dysfunction. These conditions are reflected by the amount of urinary albumin/creatinine excretion which is associated with several risk factors of the metabolic syndrome (Lee *et al.* 2006). The prevalence of microalbuminuria was 9.1% overall, and the prevalence of microalbuminuria was extremely high in girls (14.1%) which was significantly higher (p =0.000) than boys (only 4.0%) (see Figure 15). This finding is consistent with other studies that found girls to be more likely to have higher microalbuminuria than boys (Davies *et al.* 1984; Nguyen *et al.* 2008). As reported by Davies *et al.* (1984), urinary albumin excretion in girls was significantly higher daytime but not nighttime, compared with boys, in turn suggesting that girls have more orthostatic albuminuria [the condition where traces of albumin in urine are present while standing, but not recumbent] (Nguyen *et al.* 2008).

Table 5: Descriptive characteristics of demographic, hemodynamic, and renal function-related variables of SAFARI participants.

	Girls		Boys		Total	
Variable	**Sample size**	**Mean ± SD or %**	**Sample size**	**Mean ± SD or %**	**Sample size**	**Mean ± SD or %**
Age (years)	302	11.6 ± 3.5	302	11.5 ± 3.4	604	11.6 ± 3.4
Sex	302	-	302	-	604	50%
SBP (mm Hg)*	298	102.7 ± 9.2	298	105.7 ±10.1	596	104.2 ± 9.8
DBP (mm Hg)	301	63.4 ± 7.0	299	62.9 ± 7.0	600	63.1 ± 7.0
SBP≥90	302	8.9	302	11.6	604	10.3
DBP≥90	302	7.3	302	6.3	604	6.8
EBP≥90	302	11.6	302	13.6	604	12.6
MA*	297	14.1	300	4.0	597	9.1

*p <0.005 for gender, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *SBP≥90* SBP ≥90th percentile, *DBP≥90* DBP ≥90th percentile, *EBP≥90* SBP or DBP ≥90th percentile, and *MA* microalbuminuria.

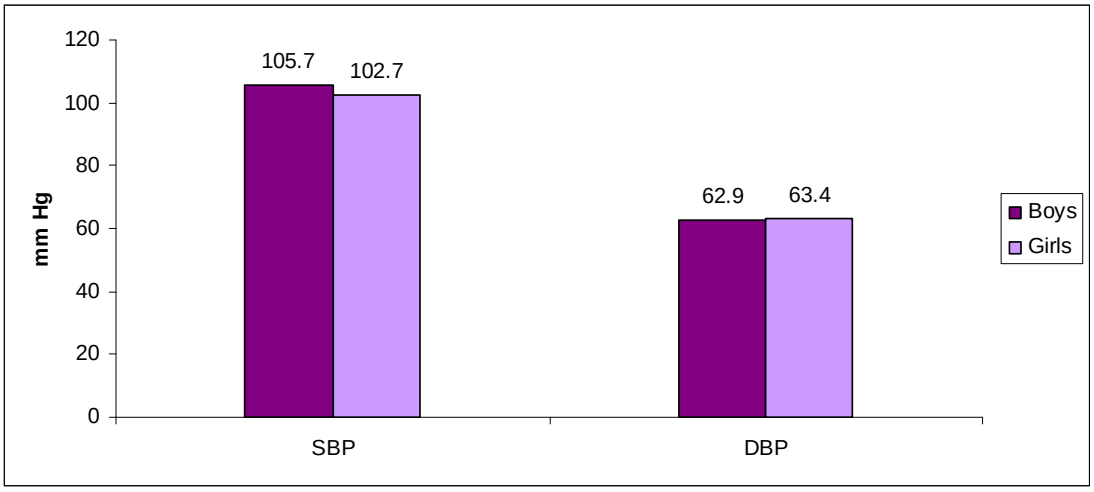

Figure 12: Systolic (boys - 105.7, girls - 102.7) and diastolic blood pressure (boys - 62.9, girls - 63.4) measures in Mexican American children by sex.

Lipid, insulin and glucose metabolism related variables

The descriptive characteristics of lipid, insulin and glucose metabolism related variables of the total study population are presented in Table 6. This table describes the variables used: total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, fasting glucose, 2-hr glucose, fasting insulin, and homeostasis model assessment of insulin resistance (HOMA-IR). The lipid profile including total cholesterol (mg/dl), triglycerides (mg/dl), HDL (mg/dl) and LDL (mg/dl) cholesterol values in boys were 149.2 ± 28.2, 75.6 ± 43.6, 45.9 ± 11.1, 87.8 ± 24.0, and in girls were 146.0 ± 24.3, 77.5 ± 38.0, 44.3 ± 9.9, 85.6 ± 20.8, respectively. Overall, the lipid measurements were higher (except for triglycerides) in boys (see Figure 13). The mean fasting glucose (mg/dl), 2-hour glucose (i.e., oral glucose tolerance test, OGTT, mg/dl) and fasting insulin (μIU/ml) measures for the total sample were 89.8 ± 7.5, 115.6 ± 21.1 and 9.0 ± 9.0, respectively. The means were significantly different for fasting glucose between boys and girls (p =0.002), and both of the glucose measurements were somewhat higher in boys (90.7 ± 7.6 and 116.2 ± 21.2) than girls (88.8 ± 7.2 and 115.1 ± 21.0), respectively. The mean HOMA-IR value in this population was 1.9 ± 1.6. The fasting insulin and HOMA-IR were higher in girls (9.7 ± 9.4 and 2.0 ± 1.7) than boys (8.4 ± 8.4 and 1.8 ± 1.4). These results indicate that the glucose measures were higher in boys, while insulin measures were higher in girls.

Dyslipidemia

The dyslipidemia pattern, characteristic to T2DM, is low HDL cholesterol levels and high triglyceride levels (i.e., hypertriglyceridemia). The prevalence of low HDL (i.e., HDL equal to or lower than 10th percentile for age and sex, Butte *et al.* 2005) in the total sample was 23.8% (boys – 21.9% and girls – 25.7%; see Figure 15). The prevalence of hypertriglyceridemia (i.e., triglycerides greater than 90th percentile for age, Butte *et al.* 2005) in the total sample was found to be 5.9% (boys – 5.7% and girls – 6.1%; see Figure 15). Thus, low HDL appears to be a major MS risk factor in SAFARI children.

Prediabetes

Impaired fasting glucose (IFG ≥100 and ≤125 mg/dl) and impaired glucose tolerance [i.e., 2 hour glucose] (IGT ≥140 and ≤199 mg/dl), or both are referred to the condition as pre-diabetes, which is a strong predictor of T2DM. The prevalence rates of IFG, IGT, and prediabetes in the total sample were 7.3%, 12.3%, and 14.7%, respectively (see Figure 15). The patterns of IFG (boys - 8%, girls - 7%) and IGT (boys - 13%, girls - 12%) appear to be similar in boys and girls. Overall, the occurrence of prediabetes in SAFARI children was about 15%.

Table 6: Descriptive characteristics of lipid, insulin and glucose metabolism related variables of SAFARI participants.

	Girls		Boys		Total	
Variable	**Sample size**	**Mean ± SD or %**	**Sample size**	**Mean ± SD or %**	**Sample size**	**Mean ± SD or %**
TC (mg/dl)	279	146.0 ± 24.3	282	149.2 ± 28.2	561	147.6 ± 26.4
TG (mg/dl)	280	77.5 ± 38.0	283	75.6 ± 43.6	563	76.5 ± 40.9
HDL (mg/dl)	279	44.3 ± 9.9	282	45.9 ± 11.1	561	45.1 ± 10.6
LDL (mg/dl)	278	85.6 ± 20.8	282	87.8 ± 24.0	560	86.8 ± 22.5
FG (mg/dl)*	282	88.8 ± 7.2	286	90.8 ± 7.7	568	89.8 ± 7.5
2-hr G (mg/dl)	194	115.1 ± 21.0	189	116.2 ± 21.2	383	115.6 ± 21.1
FI (µIU/ml)	281	9.7 ± 9.4	286	8.4 ± 8.4	567	9.0 ± 9.0
HOMA-IR	276	2.0 ± 1.7	285	1.8 ± 1.4	561	1.9 ± 1.6

*p <0.005 for gender, *TC* total cholesterol, *TG* triglycerides, *HDL* high-density lipoprotein cholesterol, *LDL* low-density lipoprotein cholesterol, *FG* fasting glucose, *2-hr G* two hour glucose, *FI* fasting insulin, and *HOMA-IR* homeostasis model assessment of insulin resistance.

Figure 13: Lipid profiles in Mexican American children by sex (boys vs. girls were *TC* total cholesterol: 149.2 vs. 146.0, *TG* triglycerides: 75.6 vs. 77.5, *HDL* high-density lipoprotein cholesterol: 45.9 vs. 44.3, and *LDL* low-density lipoprotein cholesterol: 87.8 vs. 85.6).

Adiposity and body composition measures

The adiposity and body composition measures are described in Table 7. The variables include: BMI, waist circumference, overweight, obesity, total fat mass, lean body mass, and percent body fat. The average BMI was 22.8 ± 6.6. As given in Table 7, about 52.2% of the children were overweight (BMI ≥85th percentile for age and sex) and 33.1% were obese (BMI ≥95th percentile for age and sex). The average BMI was similar for both boys and girls (22.8). However, when the BMI was dichotomized into overweight and obese categories, then 54.6% of boys tend to be at risk for overweight and 36.1% were classified as obese compared to 50.0% and 30.1% of girls indicating higher overweight and obese rates in boys. However, the prevalence of abdominal obesity (i.e. waist circumference >90th percentile for age and sex; Butte et al. 2005), was found to be 14.0%, and it was slightly higher in girls (14.8%) compared to boys (13.6%) (see Figure 15). The mean total fat mass (kg), lean body mass (kg), and percent body fat measures for the total study sample were 16.2 ± 11.2, 33.5 ± 13.6, 30.2 ± 10.0, respectively. The waist circumference (mm) and lean body mass averages were higher in boys (770.0 ± 188.9 and 35.9 ± 15.5 to 764.3 ± 174.3 and 31.0 ± 10.9), whereas the total fat mass and percent body fat were higher in girls (17.0 ± 10.9 and 32.7 ± 8.7 to 15.4 ± 11.5 and 27.8 ± 10.5).

The adiposity measures were higher for boys in all categories except for percent body fat and total fat mass given graphically in Figure 14, which is a general tendency observed in females. Whereas, the lean body mass and percent body fat measures are significantly different with p =0.000 for the gender. The sex hormones

influence the body composition differently in males and females. Males due to testosterone tend to have more bone mineral and lean muscle mass in the upper body, whereas females due to estrogen exhibit a greater percentage of body fat (Mosher and Crawford 2009).

Table 7: Descriptive characteristics of adiposity and body compoition measures of SAFARI participants.

	Girls		Boys		Total	
Variable	**Sample size**	**Mean ± SD or %**	**Sample size**	**Mean ± SD or %**	**Sample size**	**Mean ± SD or %**
BMI (kg/m^2)	302	22.8 ± 6.6	302	22.8 ± 6.5	604	22.8 ± 6.6
WC (mm)	296	764.3 ± 174.3	299	770.0 ± 188.9	595	767.2 ± 181.6
OW	302	50.0	302	54.6	604	52.2
OS	302	30.1	302	36.1	604	33.1
TF (kg)	267	17.0 ± 10.9	277	15.4 ± 11.5	544	16.2 ± 11.2
TLF (kg)*	267	31.0 ± 10.9	277	35.9 ± 15.5	544	33.5 ± 13.6
TPF *	267	32.7 ± 8.7	277	27.8 ± 10.5	544	30.2 ± 10.0

*p <0.005 for gender, *BMI* body mass index, *WC* waist circumference, *OW* overweight, *OS* obese, *TF* total fat mass, *TLF* lean body mass, *TPF* percent body fat.

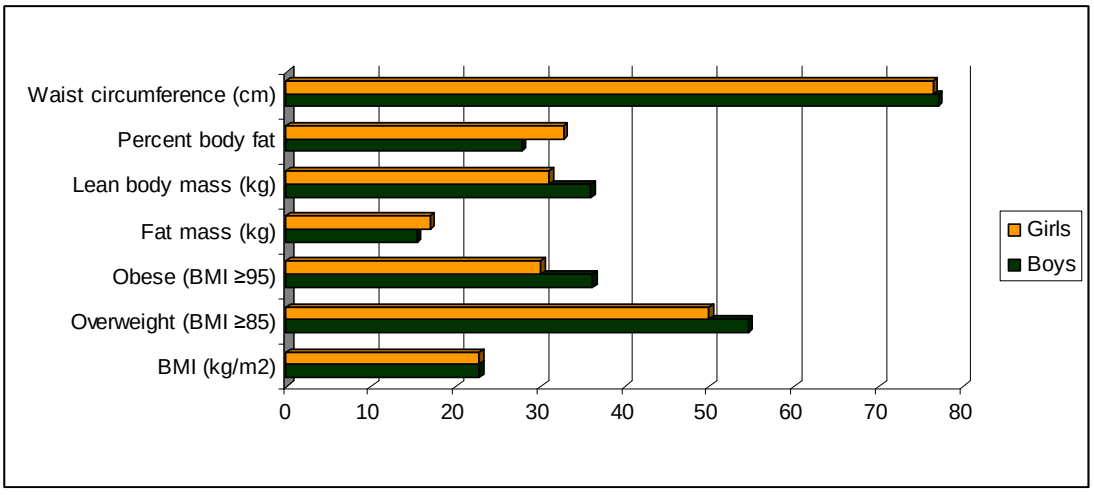

Figure 14: Adiposity measures in Mexican American children by sex (girls vs. boys were waist circumference: 76.43 vs. 77.0, percent body fat: 32.7 vs. 27.8, lean body mass: 31.0 vs. 35.9, fat mass: 17.0 vs. 15.4, obese (%): 30.1 vs. 36.1, overweight (%): 50.0 vs. 54.6, BMI: 22.8 each).

Physical activity and physical fitness

The physical activity and physical fitness scores are described in Table 8. The variables include: physical activity yesterday (METS_y), physical activity usually (METS_u), sedentary activity yesterday (SED_y), sedentary activity usually (SED_u), and physical fitness score (PFS). The physical activity and sedentary activity overall was higher than the activities from the day before data collection (2.0 ± 1.2 vs. 1.5 ± 1.2 and 0.7 ± 0.3 vs. 0.5 ± 0.3). The physical activity (including activity overall and from the day before data collection) and physical fitness score measures differ significantly for gender (p =0.000), but not the sedentary activity. The physical fitness score was 58.1 ± 31.5 representing the total study sample. The boys physical activity usually (2.2 ± 1.2 to 1.8 ± 1.2; [p =0.000]) and physical fitness scores (67.0 ± 32.5 to 49.3 ± 27.8; [p =0.000]) were significantly higher than girls indicating that boys are more likely to be physically active than girls.

Table 8: Descriptive characteristics of physical activity and physical fitness scores of SAFARI participants.

	Girls		Boys		Total	
Variable	**Sample size**	**Mean ± SD or %**	**Sample size**	**Mean ± SD or %**	**Sample size**	**Mean ± SD or %**
METS_y*	294	1.2 ± 1.1	298	1.7 ± 1.2	592	1.5 ± 1.2
METS_u*	295	1.8 ± 1.2	296	2.2 ± 1.2	591	2.0 ± 1.2
SEDS_y	295	0.5 ± 0.3	296	0.5 ± 0.3	591	0.5 ± 0.3
SEDS_u	292	0.7 ± 0.3	293	0.7 ± 0.3	585	0.7 ± 0.3
PFS*	213	49.3 ± 27.8	211	67.0 ± 32.5	424	58.1 ± 31.5

*p <0.005 for gender, *METS_y* physical activity yesterday, *METS_u* physical activity usually, *SEDS_y* sedentary activity yesterday, *SEDS_u* sedentary activity usually, *PFS* physical fitness score.

Summary of the components of metabolic syndrome

The prevalence of the components of the metabolic syndrome in Mexican American children are presented in Figure 15. In boys, higher prevalence of elevated blood pressure (13.6% to 11.6% in girls) and prediabetes (16% to 14% in girls) were observed. However, the occurrence of abdominal obesity was slightly higher in girls (14.8%) than boys (13.6%). Likewise, both low HDL and hypertriglycerdemia prevalence rates were higher in girls (25.7% and 6.1%) compared to boys (21.9% and 5.7%). In girls (14.1%), the higher prevalence of microalbuminuria (MA) was observed. In summary, these data reveal a high risk of overweight and obesity in these children – greater than 52% and 33% (Table 7), respectively. The increased occurrence of prediabetes and dyslipidemia in this cohort indicates a predisposition to diabetes-related complications. The high occurrence of microalbuminuria in these children suggests that, even at very young ages, these children may already be experiencing early signs of metabolic syndrome-related complications.

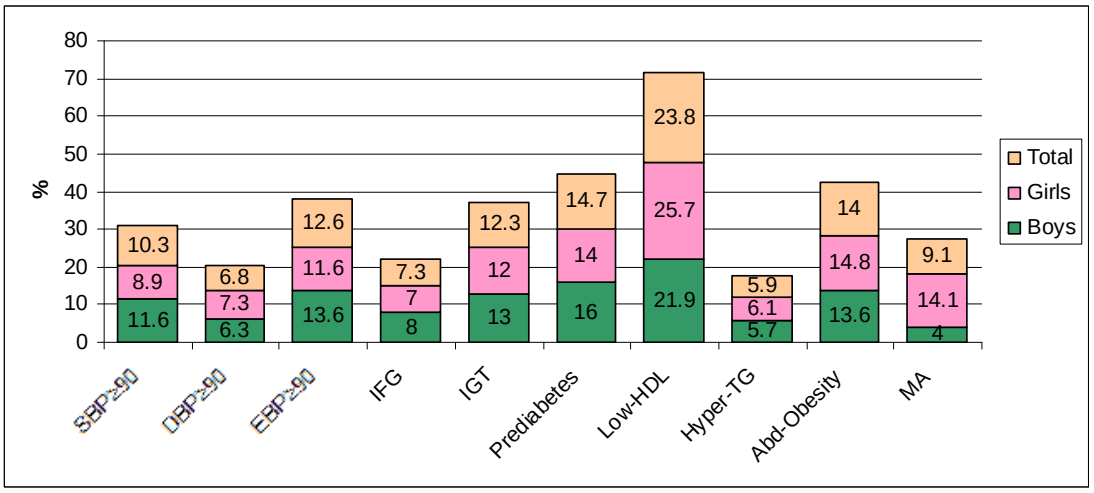

Figure 15: The prevalence of MS related components in Mexican American children. The bars represent boys at the bottom, girls in the middle, and total population at the top. *SBP≥90* SBP ≥90th percentile, *DBP≥90* DBP ≥90th percentile, *EBP≥90* SBP or DBP ≥90th percentile, *IFG* impaired fasting glucose, *IGT* impaired glucose tolerance, *Prediabetes* prediabetes, *Low-HDL* low high-density lipoprotein cholesterol, *Hyper-TG* hypertriglyceridemia, *Abd-Obesity* abdominal obesity, and *MA* microalbuminuria.

Univariate genetic analyses

The majority of variables reported in Table 9 have exhibited significant moderate-to-high heritabilities listed in Tables 5 to 8. This table provides the heritability estimates along with proportion of variance explained by significant covariates included the final models. After accounting for the variation due to age, age^2, sex, age*sex, age^2*sex, puberty, physical activity and fitness, the heritability estimates range from 25% (2-hr glucose) to 100% for DBP≥90 and percent body fat. The high heritabilities observed for various traits should be interpreted with caution since the analytical design used in this study did not allow for shared environmental effects. Thus, given the nature of this pediatric population (i.e., 6-17 years old children living with their parents), the estimates of heritability are potentially inflated

due to shared familial-environmental influences. However, such estimates are reflective of increased familial aggregation of these traits.

The blood pressure variables including both continuous (SBP and DBP - 70%) and dichotomous (SBP≥90 - 97% and BP≥90 - 76%) were highly heritable in this SAFARI cohort. Likewise, high heritabilities were observed for various traits, especially the overweight and obesity measures (84% for both) followed by total fat mass (79%), triglycerides (78%), HDL (69%), BMI and waist circumference (each 66%), respectively. Subsequently, high-to-moderate heritabilities were observed for HOMA-IR (55%), total cholesterol (54%), LDL and microalbuminuria (each 49%), fasting insulin (45%) and fasting glucose (27%). However, heritability estimates of all the traits were significantly (p <0.05) greater than zero.

Table 9: Heritabilities of MS-related phenotypes with proportion of variance and significant covariates in the model.

Phenotype	Heritability ± S.E (*p* value)	Proportion of Variance	Significant Covariates
SBP (mm Hg)	**0.70 ± 0.11 (<0.0001)**	18.2%	Age, Sex, Age*Sex
DBP (mm Hg)	**0.70 ± 0.15 (<0.0001)**	8.9%	Age, Puberty, PFS
SBP≥90	**0.97 ± 0.27 (0.0005)**	-	
DBP≥90	**1.00** [b] **(0.0008)**	-	PFS
EBP≥90	**0.76 ± 0.37 (0.0216)**	-	PFS
MA	**0.49 ± 0.11 (0.0233)**	6%	Sex
TC (mg/dl)	**0.54 ± 0.16 (0.0004)**	6.3%	Age, Puberty, PFS
TG (mg/dl) [a]	**0.78 ± 0.14 (<0.0001)**	5.9%	Age*Sex, Age^2 , PFS
HDL (mg/dl)	**0.69 ± 0.15 (<0.0001)**	6.8%	Age*Sex, Puberty, PFS
LDL (mg/dl)	**0.49 ± 0.17 (0.0015)**	5.8%	Age, Age*Sex, Puberty, PFS
FG (mg/dl)	**0.27 ± 0.12 (0.0080)**	0.6%	Puberty
2-hr G (mg/dl)	**0.25 ± 0.15 (0.0391)**	0.4%	Age^2
FI (µIU/ml) [a]	**0.45 ± 0.16 (0.0012)**	15.7%	Age^2, Puberty
HOMA-IR [a]	**0.55 ± 0.17 (0.0003)**	14.9%	Puberty, PFS
BMI (kg/m^2)[a]	**0.66 ± 0.14 (<0.0001)**	37.4%	Age, Sex, Age^2 , Puberty, PFS
WC (mm)	**0.66 ± 0.14 (<0.0001)**	39.0%	Age, Sex, Age*Sex, Age^2, Puberty, PFS
OW	**0.84 ± 0.23 (0.0002)**	7.0%	Sex, Age^2, Puberty, PFS
OS	**0.84 ± 0.23 (0.0009)**	10.0%	Sex, Age*Sex, Age^2, Puberty, PFS
TF (kg) [a]	**0.79 ± 0.15 (<0.0001)**	44.9%	Age, Age^2, Puberty, PFS
TLF (kg) [a]	**0.57 ± 0.16 (0.0003)**	-	
TPF	**1.00**[b] **(<0.0001)**	19.5%	Age*Sex, Age^2, PFS

[a] Log transformed, [b] Went to the boundary, $p \leq 0.05$ are considered significant and in bold, *SE* standard error, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *SBP ≥90* SBP ≥ 90^{th} percentile, *DBP≥90* DBP ≥ 90^{th} percentile, *EBP≥90* SBP or DBP ≥ 90^{th} percentile, *PFS* physical fitness score, *MA* microalbuminaria, *TC* total cholesterol, *TG* triglycerides, *HDL* high-density lipoprotein cholesterol, *LDL* low-density lipoprotein cholesterol, *FG* fasting glucose, *2-hr G* two hour glucose, *FI* fasting insulin, *HOMA-IR* homeostasis model assessment for insulin resistance, *BMI* body mass index, *WC* waist circumference, *OW* overweight, *OS* obese, *TF* total fat mass, *TLF* lean body mass, *TPF* percent body fat.

When the covariates were concerned (excluding the questionnaire-based physical activity measures which showed no effect on any traits), age, age^2, puberty and physical fitness score reveal significant effects on total fat mass explaining about 45% variation in the phenotype. For waist circumference, 39% of variation in the trait was significantly influenced by age, sex, age*sex, age^2, puberty, fitness score. Similarly for BMI, 37.4% of variation is explained by all of these covariates except age*sex. For overweight and obese, about 7 -10% of phenotypic variation was explained by these environmental covariates. With HOMA-IR and insulin measures, puberty and fitness score had significant effects with proportion of variance ranged from 14.9 - 16.7%. For glucose measures including both fasting and 2-hr OGTT, age^2 and puberty were found to be significant covariates. In case of lipids, ~6 - 7% of variations in the traits were explained significantly by age, puberty, fitness score for total cholesterol, all of these covariates along with age*sex for LDL, age*sex, puberty, fitness score for HDL, and for triglycerides – age^2 instead of puberty. For microalbuminuria, only sex effects (6%) were found to be a significant covariate. For the blood pressure variables, age, sex and age*sex had significant effects (18.2%) on systolic blood pressure trait. But for diastolic blood pressure, it was age, puberty and fitness score (8.9%), and just fitness score for hypertension, a dichotomous variable.

Multivariate genetic analyses

Systolic blood pressure (SBP) as a quantitative trait and other MS-related traits

The phenotypic, additive genetic, and random environmental correlations between SBP (as a continuous variable) and metabolic syndrome-related traits are presented in Table 10. The phenotypic correlations were statistically significant (p <0.05) for most of the variables considered, with the exception of microalbuminuria, HDL and LDL cholesterol, ranged from 0.13 to 0.31. While, the total cholesterol showed borderline significant phenotypic correlation (0.09, p =0.0880). The genetic correlations were significant for triglycerides, fasting insulin, HOMA-IR, BMI, waist circumference, overweight, and lean body mass and ranged from 0.32 to 0.53. For the traits such as obese and total fat mass there was suggestive evidence for significant genetic correlation. There were no significant environmental correlations for SBP with other traits, and in general, these correlations were substantially lower than the genetic correlations. Therefore, the phenotypic correlations of SBP with metabolic syndrome-related traits are mostly influenced by genetic factors but not the environmental factors. Thus, pleiotropy (i.e., common genetic influences on correlated traits) appears to underlie the observed clustering of SBP with other important components of the MS.

Table 10: Phenotypic (ρ_P), genetic (ρ_G), and environmental(ρ_E) correlations between systolic blood pressure (SBP) and other MS risk factors (SBP as a continuous variable; ρ correlation, SE standard error, *HDL* high-density lipoprotein cholesterol, *LDL* low-density lipoprotein cholesterol, *HOMA-IR* homeostasis model assessment of insulin resistance, *BMI* body mass index).

Trait	$\rho_P \pm$ SE (*p* value)	$\rho_G \pm$ SE (*p* value)	$\rho_E \pm$ SE (*p* value)
Microalbuminuria	-0.03 ± 0.08 (0.7281) ns	-0.53 ± 0.43 (0.0982) ns	0.59 ± 0.33 (0.0630) ns
Total cholesterol (mg/dl)	0.09 ± 0.05 (0.0880)*	0.22 ± 0.20 (0.2597) ns	-0.13 ± 0.27 (0.6318) ns
Triglycerides (mg/dl)[a]	**0.19 ± 0.05 (0.0001)**	**0.32 ± 0.15 (0.0395)**	-0.22 ± 0.37 (0.5360) ns
HDL (mg/dl)	-0.07 ± 0.05 (0.1722) ns	-0.04 ± 0.17 (0.8212) ns	-0.15 ± 0.32 (0.6425) ns
LDL (mg/dl)	0.08 ± 0.05 (0.1117) ns	0.15 ± 0.20 (0.4638) ns	-0.02 ± 0.26 (0.9439) ns
Fasting glucose (mg/dl)	**0.15 ± 0.04 (0.0007)**	0.08 ± 0.21 (0.6861) ns	0.24 ± 0.17 (0.1732) ns
2-hr glucose (mg/dl)	**0.13 ± 0.05 (0.0194)**	0.13 ± 0.27 (0.6451) ns	0.16 ± 0.20 (0.4395) ns
Fasting insulin (µIU/ml)[a]	**0.25 ± 0.05 (<0.0001)**	**0.53 ± 0.20 (0.0073)**	-0.09 ± 0.24 (0.6982) ns
HOMA-IR[a]	**0.27 ± 0.06 (<0.0001)**	**0.44 ± 0.20 (0.0306)**	-0.02 ± 0.30 (0.9530) ns
BMI (kg/m^2)[a]	**0.29 ± 0.05 (<0.0001)**	**0.39 ± 0.15 (0.0136)**	0.04 ± 0.29 (0.8870) ns
Waist circumference (mm)	**0.27 ± 0.05 (<0.0001)**	**0.34 ± 0.15 (0.0369)**	0.10 ± 0.28 (0.7182) ns
Overweight	**0.31 ± 0.06 (<0.0001)**	**0.49 ± 0.18 (0.0087)**	-0.30 ± 0.66 (0.5878) ns
Obese	**0.29 ± 0.06 (<0.0001)**	0.35 ± 0.20 (0.0808)*	0.06 ± 0.50 (0.9067) ns
Total fat (kg)[a]	**0.29 ± 0.05 (<0.0001)**	0.27 ± 0.15 (0.0854)*	0.31 ± 0.35 (0.4163) ns
Total lean fat (kg)[a]	**0.28 ± 0.04 (<0.0001)**	**0.33 ± 0.15 (0.0456)**	0.21 ± 0.21 (0.3499) ns
Total percent fat	**0.24 ± 0.04 (<0.0001)**	0.19 ± 0.12 (0.1306) ns	1.00[b] (0.4409) ns

[a]Log transformed, [b]Went to the boundary, *Suggestive evidence for correlation, Significant correlations with $p \leq 0.05$ are represented in bold, ns Not significant

Diastolic blood pressure (DBP) as a quantitative trait and other MS-related traits

The phenotypic, additive genetic, and random environmental correlations between DBP (as a continuous variable) and other metabolic syndrome risk factors are presented in Table 11. The phenotypic correlations were statistically significant (p <0.05) for most of the variables considered, with the exception of microalbuminuria, total cholesterol, HDL, LDL, and 2-hr glucose, ranged from 0.12 to 0.22. While, the fasting glucose showed borderline significant phenotypic correlation (0.09, p =0.0939). The genetic correlations were significant for triglycerides, fasting insulin, HOMA-IR, BMI, overweight, obese, total fat mass and percent body fat and ranged from 0.32 to 0.61. For the traits such as HDL and waist circumference there was suggestive evidence for borderline significant genetic correlations -0.31 and 0.32, respectively. The environmental correlation was statistically significant only for triglycerides (-0.77) and suggestive evidence was observed for obese measure. Since that the genetic correlation between DBP and triglycerides was strong and positive, the estimated large standard error for the negative environmental correlation between the same traits downplays its significance. Overall, as in the case of SBP, the clustering of DBP with other MS-related traits is largely attributable to the common genetic influences (i.e., pleiotropy).

Table 11: Phenotypic (ρ_P), genetic (ρ_G), and environmental(ρ_E) correlations between diastolic blood pressure (DBP) and other MS risk factors (DBP as a continuous variable; ρ correlation, SE standard error, *HDL* high-density lipoprotein cholesterol, *LDL* low-density lipoprotein cholesterol, *HOM-IR* homeostasis model assessment of insulin resistance, *BMI* body mass index).

Trait	$\rho_P \pm$ SE (*p* value)	$\rho_G \pm$ SE (*p* value)	$\rho_E \pm$ SE (*p* value)
Microalbuminuria	0.03 ± 0.09 (0.7542) ns	0.53 ± 0.34 (0.0735) ns	0.78 ± 0.38 (0.0253) ns
Total cholesterol (mg/dl)	0.08 ± 0.05 (0.1398) ns	0.05 ± 0.20 (0.8001) ns	0.10 ± 0.28 (0.7315) ns
Triglycerides (mg/dl)[a]	**0.15 ± 0.05 (0.0035)**	**0.49 ± 0.16 (0.0027)**	**-0.77 ± 0.48 (0.0400)**
HDL (mg/dl)	-0.07 ± 0.05 (0.1523) ns	-0.31 ± 0.17 (0.0773)*	0.46 ± 0.39 (0.1819) ns
LDL (mg/dl)	0.08 ± 0.05 (0.1179) ns	0.10 ± 0.21 (0.6293) ns	0.03 ± 0.27 (0.8973) ns
Fasting glucose (mg/dl)	0.09 ± 0.05 (0.0939)*	0.21 ± 0.24 (0.3767) ns	-0.00 ± 0.21 (0.9835) ns
2-hr glucose (mg/dl)	0.09 ± 0.06 (0.1467) ns	0.21 ± 0.28 (0.4546) ns	-0.02 ± 0.23 (0.9168) ns
Fasting insulin (µIU/ml)[a]	**0.22 ± 0.05 (0.0003)**	**0.55 ± 0.24 (0.0163)**	-0.18 ± 0.26 (0.4754) ns
HOMA-IR[a]	**0.20 ± 0.06 (0.0007)**	**0.52 ± 0.22 (0.0169)**	-0.29 ± 0.30 (0.3139) ns
BMI (kg/m^2)[a]	**0.20 ± 0.05 (<0.0001)**	**0.39 ± 0.16 (0.0225)**	-0.21 ± 0.32 (0.4945) ns
Waist circumference (mm)	**0.18 ± 0.05 (0.0004)**	0.32 ± 0.17 (0.0676)*	-0.13 ± 0.32 (0.6914) ns
Overweight	**0.20 ± 0.06 (0.0020)**	**0.48 ± 0.20 (0.0164)**	-0.71 ± 0.74 (0.2281) ns
Obese	**0.19 ± 0.07 (0.0053)**	**0.61 ± 0.27 (0.0095)**	-0.87 ± 0.66 (0.0958)*
Total fat (kg)[a]	**0.21 ± 0.05 (0.0002)**	**0.42 ± 0.16 (0.0145)**	-0.39 ± 0.47 (0.3351) ns
Total lean fat (kg)[a]	**0.12 ± 0.05 (0.0227)**	0.10 ± 0.19 (0.6006) ns	0.11 ± 0.25 (0.6586) ns
Total percent fat	**0.19 ± 0.05 (<0.0001)**	**0.32 ± 0.14 (0.0003)**	-1.00[b] (0.3722) ns

[a]Log transformed, [b]Went to the boundary, *Suggestive evidence for correlation, Significant correlations with $p \leq 0.05$ are represented in bold, ns Not significant

Elevated blood pressure (EBP) as a dichotomous trait and other MS-related traits

The phenotypic, additive genetic, and random environmental correlations between EBP (as a dichotomous variable) and other metabolic syndrome risk factors are presented in Table 12. The results are similar to those presented in Tables 10 and 11. The phenotypic correlations were statistically significant (p <0.05) for all the variables considered, with the exception of total cholesterol, LDL, and lean body mass, ranged from 0.21 to 0.34 (excluding HDL for which the phenotypic correlation was -0.24). The genetic correlation was significant only for triglycerides (0.51). Given the observed patterns of genetic correlations between SBP and DBP and other MS-related traits, the inability to find significant genetic correlations between EBP, a dichotomized trait, and other MS-related traits is attributable to power loss for genetic analysis due to dichotomization of a continuous trait (Duggirala et al. 1997). The fact that the patterns of phenotypic correlations were similar to SBP and DBP and significant, increased sample sizes could help to partition the observed significant phenotypic correlations into genetic and environmental correlations efficiently.

Table 12: Phenotypic (ρ_P), genetic (ρ_G), and environmental(ρ_E) correlations between elevated blood pressure [EBP] (SBP or DBP ≥90th percentile) and other MS risk factors (EBP as a dichotomous variable; ρ correlation, SE standard error, *HDL* high-density lipoprotein cholesterol, *LDL* low-density lipoprotein cholesterol, *HOMA-IR* homeostasis model assessment of insulin resistance, *BMI* body mass index).

Trait	ρ_P ± SE (*p* value)	ρ_G ± SE (*p* value)	ρ_E ± SE (*p* value)
Microalbuminuria	**0.23 ± 0.11 (0.0376)**	-0.29 ± 0.51 (0.5116) ns	0.89 ± 0.83 (0.2163) ns
Total cholesterol (mg/dl)	0.07 ± 0.08 (0.3821) ns	0.11 ± 0.29 (0.7251) ns	-0.01 ± 0.50 (0.9823) ns
Triglycerides (mg/dl)[a]	**0.23 ± 0.08 (0.0041)**	**0.51 ± 0.25 (0.0459)**	-0.65 ± 0.96 (0.3731) ns
HDL (mg/dl)	**-0.24 ± 0.08 (0.0073)**	-0.21 ± 0.29 (0.4698) ns	-0.32 ± 0.64 (0.6364) ns
LDL (mg/dl)	0.11 ± 0.08 (0.2027) ns	0.08 ± 0.32 (0.8078) ns	0.17 ± 0.55 (0.7701) ns
Fasting glucose (mg/dl)	**0.23 ± 0.08 (0.0086)**	-0.25 ± 0.44 (0.5349) ns	0.71 ± 0.47 (0.0571)*
2-hr glucose (mg/dl)	**0.21 ± 0.10 (0.0420)**	0.45 ± 0.46 (0.3447) ns	0.05 ± 0.51 (0.9330) ns
Fasting insulin (µIU/ml)[a]	**0.33 ± 0.10 (0.0020)**	0.28 ± 0.34 (0.4038) ns	0.41 ± 0.45 (0.3525) ns
HOMA-IR[a]	**0.30 ± 0.10 (0.0035)**	0.31 ± 0.30 (0.3260) ns	0.28 ± 0.52 (0.5990) ns
BMI (kg/m^2)[a]	**0.31 ± 0.08 (<0.0001)**	0.07 ± 0.66 (0.7655) ns	1.00[b] (0.1223) ns
Waist circumference (mm)	**0.32 ± 0.07 (<0.0001)**	0.31 ± 0.24 (0.2453) ns	0.34 ± 0.57 (0.5739) ns
Overweight	**0.34 ± 0.10 (0.0017)**	0.15 ± 1.00 (0.5602) ns	1.00[b] (0.2455) ns
Obese	**0.29 ± 0.10 (0.0069)**	0.51 ± 0.38 (0.1440) ns	-0.43 ± 1.08 (0.6733) ns
Total fat (kg)[a]	**0.30 ± 0.08 (0.0058)**	0.20 ± 0.26 (0.4584) ns	0.63 ± 0.84 (0.4575) ns
Total lean fat (kg)[a]	0.12 ± 0.09 (0.1583) ns	0.24 ± 0.34 (0.4745) ns	-0.11 ± 0.58 (0.8460) ns
Total percent fat	**0.22 ± 0.08 (0.0006)**	0.16 ± 0.85 (0.4015) ns	1.00[b] (0.4307) ns

[a]Log transformed, [b]Went to the boundary, *Suggestive evidence for correlation, Significant correlations with $p \leq 0.05$ are represented in bold, ns Not significant

CHAPTER FIVE: DISCUSSION

Childhood obesity is associated with various metabolic abnormalities, and it increases the risk of adult obesity and its correlated diseases. For example, childhood obesity is a leading cause of pediatric hypertension (Sorof and Daniels 2002; Din-Dzietham *et al.* 2007; Forrest and Leeds 2007; Genovesi *et al.* 2008; Ostchega *et al.* 2009). However, there is limited information about the genetic influences on childhood elevated blood pressure as well as potential common genetic influences on blood pressure and various metabolic syndrome-related traits (e.g., obesity). Therefore, using a Mexican American Children cohort, the major goal of this study is to address the following issues: (1) examination of various metabolic syndrome-related traits in Mexican American children; (2) determination of genetic influences on blood pressure measures; and (3) verification of common genetic influences affecting both blood pressure and metabolic syndrome-related traits by partitioning their phenotypic correlations into genetic and environmental components.

Although the metabolic syndrome and its components (e.g., high blood pressure profiles) have been well established for adults, there has been no consensus about such criteria (i.e., cut points to define MS risk factors) in children and adolescents. As stated by Steinberger *et al.* (2009:629), such MS criteria in children-based studies, "have been variably adapted from adult standards with the use of age- and gender-dependent normal values". Given the paucity of data on MS components in normal Mexican American children, this study used a similar approach (i.e., percentiles for age, sex, and height [in the case of blood pressure only]) to define high

blood pressure and other MS risk factors. The results of this study indicate that the occurrence of various MS risk factors in normal Mexican American children aged 6-17 years is rather disturbing (Table 7 & Figure 15; e.g., overweight = 52%, obesity = 33%, low HDL = 24%, prediabetes = 15%, increased abdominal obesity = 14%, and high blood pressure = 13%).

The prevalence rates of various MS risk factors examined in this study varied by gender. In general, boys tended to have higher prevalence rates of various MS risk factors compared to girls. For example, the prevalence rates of systolic blood pressure, diastolic blood pressure, and elevated blood pressure varied between the sexes; especially boys have a higher prevalence of hypertension. Similarly, when the BMI is dichotomized, the prevalence of both overweight and obesity are higher in boys than girls, although girls exhibited slightly increased occurrence of abdominal obesity based on waist circumference. A high prevalence of overweight was observed in Mexican American adolescents, especially in males. Hypertension is observed in these overweight children, even after adjustment for other risk factors (Cossrow and Falkner 2004; Forrest and Leeds 2007).

The occurrence of dyslipidemia (i.e., low HDL and hypertriglycerdemia) is slightly increased in girls compared to boys. Whereas, the rates of impaired fasting glucose, impaired glucose tolerance, prediabetes are found to be slightly increased in boys compared to girls. However, the patterns of fasting insulin and HOMA-IR (a measure of insulin resistance which is inversely related to insulin sensitivity) slightly varied by gender with an indication that girls are more insulin resistant (high fasting

insulin and HOMA-IR values) than boys as were validated by other studies (Cruz *et al*. 2004). In general, Hispanic children compared with non-Hispanic whites had significantly higher levels of fasting insulin and are more insulin resistant than Caucasian children (Goran *et al.* 2003). The adiposity measures (except total fat mass and percent body fat) are higher in boys than girls. Increase in adiposity over time is significantly associated with increased blood pressure levels (Jafar 2009). Similarly, insulin resistance is correlated with high blood pressure (Ferrannini *et al.* 1987; Steinberger *et al.* 2009). Although boys appear to be physically active and fit based on activity and fitness scores, they are at higher risk for metabolic syndrome-related chronic conditions.

Microalbuminuria is a measure of renal function impairment and an early marker of diabetic nephropathy. It is also considered as a biological marker that identifies individuals who are at higher risk for metabolic syndrome-related conditions. In this study, its occurrence is more in girls than boys. Earlier studies confirm the existence of strong correlations between blood pressure, serum glucose and microalbuminuria (Jones *et al.* 2002; Lee *et al.* 2006). Since microalbuminuria clusters in families, a genetic predisposition is hypothesized. Using genome-wide linkage analysis (after accounting for age, sex, BMI, triglycerides, and hypertension), a major locus was located on chromosome 20q12 that may have functional relevance to albumin excretion in Mexican Americans (Arar *et al.* 2007).

The few studies in the past years that have examined the secular trend in blood pressure have yielded inconsistent results. The Minneapolis blood pressure study on public school children including African Americans, Hispanics, non-Hispanic whites, Native Americans, and Asians aged 10-14 years found a significant increase in systolic blood pressure percentiles than for diastolic blood pressure from 1986 to 1996. In the Bogalusa study on two biracial cohorts [~65% white and 35% black] aged 7 to 9 years. One cohort examined in 1975 and reexamined in 1981; second cohort in 1984 and reexamined in 1992 and both were compared, both systolic and diastolic blood pressure decreased by the end of study period. Another study based on boys and girls (representing non-Hispanic black/white or Mexican American ethnicity) aged 8 to 17 years participated in national surveys from 1963 to 2002 verified increasing blood pressure trends in children. After a long period of decreased blood pressure trend, since the late 1980s an elevated blood pressure (observed SBP or DBP $\geq 95^{th}$ percentile) and pre-elevated blood pressure (observed SBP or DBP $\geq 90^{th}$ percentile but $< 95^{th}$ percentile) increase in US children and adolescents was observed. In particular, rapid rise was seen in diastolic blood pressure when compared to systolic blood pressure. Mexican Americans have a higher prevalence of elevated and pre-elevated blood pressure than non-Hispanic whites. Males have a greater prevalence than females (Gidding *et al.* 1995; Luepker *et al.* 1999; Din-Dzietham *et al.* 2007). These results are similar to the findings observed in the current SAFARI participants.

The comparative data shown in Table 13, describes study populations (overweight children) of which 71% represent Mexican Americans from Los Angeles who are of Hispanic origin and have a family history of T2DM (Cruz *et al.* 2004). About 82% of subjects had BMI $\geq 95^{th}$ percentile for age and gender, and ~90% of the participants had at least one of the components of the metabolic syndrome which includes abdominal obesity, low HDL cholesterol, hypertension, hypertriglyceridemia, and impaired glucose tolerance. For example, in this study, boys had significantly higher fasting blood glucose and systolic blood pressure, similar to the present findings of SAFARI data. In contrast, these boys exhibited significantly higher diastolic blood pressure than did the girls. Overall, the insulin sensitivity is lower in SAFARI participants than these children.

Table 13. Descriptive characteristics of overweight Hispanic boys and girls for comparison to present study sample (adapted from Cruz *et al.* 2004).

	Boys (n = 73)	Girls (n = 53)	Total (n = 126)
Age (yr)	11.0 ± 1.7	10.7 ± 1.8	10.9 ± 1.7
Height (cm)	149.2 ± 10.9	147.4 ± 12.3	148.4 ± 11.5
Weight (kg)	62.8 ± 17.2	63.8 ± 22.7	63.2 ± 19.6
Tanner	1.8 ± 1.1[a]	2.8 ± 1.4	2.2 ± 1.3
BMI (kg/m^2)	27.7 ± 4.7	28.6 ± 7.1	28.1 ± 5.8
BMI percentile	97.3 ± 2.9	97.1 ± 3.0	97.2 ± 2.9
Waist circumference (cm)	88.9 ± 11.4	85.9 ± 14.5	87.7 ± 12.8
Total fat mass (kg)	23.2 ± 8.4	25.0 ± 10.9	24.0 ± 9.6
Total lean tissue mass (kg)	37.3 ± 9.5	35.3 ± 10.8	36.4 ± 10.8
Fasting glucose (μU/ml)	93.5 ± 6.1[b]	90.1 ± 7.9	92.1 ± 7.1
2-h Glucose (mg/dl)	127.0 ± 19.0	125.5 ± 16.4	126.4 ± 17.9
Fasting insulin (μU/ml)	19.0 ± 11.1	19.2 ± 10.3	19.1 ± 10.8
Insulin sensitivity [$\times 10^{-4}$ $min^{-1}/(\mu U/ml)$]	2.01 ± 1.12	2.24 ± 1.52	2.10 ± 1.30
Acute insulin response [(μU/ml × 10 min]	1873 ± 164	1561 ± 158	1742 ± 117
Systolic blood pressure (mm Hg)	111 ± 11	109 ± 10	110 ± 11
Diastolic blood pressure (mm Hg)	62 ± 6[c]	60 ± 5	61 ± 6
Cholesterol total (mg/dl)	159.2 ± 26.8	153.0 ± 26.9	156.6 ± 26.9
LDL cholesterol (mg/dl)	95.4 ± 22.6	92.4 ± 22.1	94.2 ± 22.4
HDL cholesterol (mg/dl)	38.1 ± 8.9	39.1 ± 8.2	38.5 ± 8.6
Triglycerides (mg/dl)	128.4 ± 74.2	106.2 ± 46.1	119.1 ± 64.6

To examine the relationship between blood pressure and metabolic syndrome-related traits across ethnic groups, the results of the present study are compared with the data (given in Table 14) available from literature on black and white children (Cruz *et al.* 2002). They found that black children had significantly higher systolic and diastolic blood pressure values than white children. Black children had lower insulin sensitivity than white children and there were no significant differences in fasting insulin or fasting glucose measures. The study indicated that insulin sensitivity was significantly related to systolic blood pressure in both blacks and whites; hence ethnicity and insulin resistance were independently related to hypertension even at early ages. Boys had significantly higher diastolic blood pressure than girls, whereas, girls had higher total fat mass, percent body fat and fasting insulin values than boys; this finding is consistent with the present study. The systolic blood pressure for SAFARI children is almost similar to the whites, but the diastolic is higher than these two ethnic groups. Similarly, the adiposity measures are higher compared to these two groups. Another comparative study by Goran *et al.* (2002) on Caucasian, African-American and Hispanic youth in Los Angeles found that Hispanic children have lower insulin sensitivity than Caucasians and almost similar to African-Americans. The findings of the present study signify the increasing burden of disease risk in SAFARI children due to elevated blood pressure, higher prevalence of overweight and obesity, and lower insulin sensitivity.

Table 14. Descriptive characteristics by gender and ethnic group to compare with SAFARI sample (adapted from Cruz *et al*. 2002).

	Whites (n=58)			Blacks (n=43)		
	Boys	Girls	Total	Boys	Girls	Total
Age, y	9.5±1.2	9.5±1.2	9.5±1.2	8.9±1.3	9.4±1.2	9.1±1.3
Height, cm	137.8±9.1	136.0±9.3	136.9±9.2	137.1±9.7	136.9±10.3	137.0±9.9
Weight, kg	38.1±10.6	37.5±13.8	37.8±12.2	37.0±10.5	36.0±12.1	36.5±11.2
Total fat mass, kg	10.1±6.9	11.2±8.8	10.6±7.9	9.1±7.0	10.4±7.2	9.8±7.1
Total lean mass, kg	25.2±41.1	24.0±54.4	24.6±48.2	25.9±43.1	23.7±52.5	24.8±48.6
% Body fat	24.8±11.6	27.7±10.8	26.3±11.2	22.4±11.4	26.7±9.8	24.5±10.7
Fasting insulin, pmol/L	75.7±27.8	89.6±62.5	82.6±48.6	84.7±49.3	92.4±50.7	88.2±50.0
Fasting glucose, mmol/L	94.5±6.0	92.4±4.7	93.5±5.5	94.5±5.7	93.2±6.8	93.9±6.2
Insulin sensitivity, $\times 10^{-4} \cdot min^{-1} \cdot$ pmol/L*	55.6±31.3	49.3±33.3	52.8±31.9	36.1±16.7	27.8±18.8	31.9±18.1
Acute insulin response, pmol/L×10 min†	4237±2501	5007±4049	4625±3361	10 605±5445	11 105±5021	10 848±5188
Systolic blood pressure, mm Hg‡	106±9	104±8	105±9	111±9	109±9	110±9
Diastolic blood pressure, mm Hg‡§	57±7	52±8	54±8	60±7	58±7§	59±7

Univariate genetic analyses

It is evident from the above discussion that, although the MS risk factor profiles are varied by ethnicity possibly indicating population genetic differences, they are often found to be correlated (e.g., obesity and blood pressure). As described previously, in this study, blood pressure measures exhibited significant phenotypic correlations with various MS-related traits including lipid, obesity, insulin and glucose metabolism related traits. The components of metabolic syndrome are complex disease conditions resulting from genetic and environmental factors influences and their interactions. Also, several genes are presumably involved in the expression of a given phenotype. For example, a number of genes are involved in the pathways of insulin action; hence, any genetic defect(s) in these pathways might contribute to insulin resistance. Likewise, any genetic defect(s) relating to the genes involved in pathways regulating fluid retention by kidneys and blood pressure could contribute to hypertension.

It is well established that the MS-related traits in adults such as obesity, T2DM, and hypertension have genetic determinants (Duggirala *et al.* 2000a; Pankow *et al.* 2001; Comuzzie 2002; Stern 2002; Elbein 2002; Loos and Bouchard 2003; Lin *et al.* 2006). In nondiabetic Mexican American family members, there is evidence that factor structures clustering the metabolic syndrome have strong genetic influences (Duggirala *et al.* 2000a, 2001). Likewise, this pediatric study reports strong genetic influence on MS-related phenotypes. The heritabilities of systolic blood pressure and diastolic blood pressure (as a continuous variable), and elevated

blood pressure (as a dichotomous variable) are very high in this population, suggesting that genetic factors influence blood pressure. The high heritability estimates (~ 25 to 85%) are observed including almost all of the variables that have been examined. This genetic component of variance is attributable to additive polygenic effects and probably results from the action of more than one gene. It should be noted that, in contrast to the adult studies, the MS-related traits examined in children to estimate trait-specific heritability are not influenced by traditional MS-related covariates such as smoking and alcoholism. As noted previously, the observed heritability estimates in this study, after accounting for the significant covariate effects, are somewhat inflated since the methodological design in this study did not allow for estimation of shared environmental influences. However, as reported in Appendix 3, it is interesting to note that the influence of shared environmental influences on adult MS-related traits in Mexican Americans appears to be minimal. Also, in this study, the estimates of genetic variance were attributable to additive genetic influences, and no attempts were made to estimate the influences of dominance and epistasis.

Aside from genetic influences, the changing environmental factors such as poor dietary habits, reduced physical activity, and increased television viewing time leading to positive energy balance and significantly contribute to the increased prevalence rates of obesity- and metabolic syndrome-related phenotypes (Harper 2006; Mehta 2007; Gable *et al.* 2007; Moore *et al.* 2008). With regard to covariate effects, as such physical activity showed no effect on any traits, conversely pubertal

status and physical fitness scores along with age and sex effects significantly contributed to the phenotypic variation of many traits considered for this study. Perhaps, the questionnaire-based physical activity measures have limitations to evaluate physical activity patterns efficiently. On the other hand, increased physical activity and fitness could lead to favorable lipid profiles and improvement in insulin sensitivity (Isomaa 2003; CDC 2004) which is indicated in this study as physical fitness score clearly affects the lipid phenotypes such as total cholesterol, triglycerides, HDL and LDL cholesterol, and insulin measures. Another potential contributing factor to the development of insulin resistance in children is puberty (ADA 2000; Goran *et al.* 2003), which is supported in this study as pubertal status significantly affected the insulin and glucose measures in this children cohort.

Multivaraite genetic analyses

Since the clustering of MS-related phenotypes could be due the common genetic influences (i.e., pleiotropy), in contrast to a number of population-based pediatric studies (discussed below), the phenotypic, genetic and environmental correlations of blood pressure were examined after adjusting for trait-specific covariate effects and the phenotypic correlations were partitioned into genetic and environment (nongenetic) components in this family-based children cohort. The systolic blood pressure had significant genetic correlations with metabolic syndrome-related risk factors, especially fasting insulin, overweight, HOMA-IR, BMI, waist circumference, lean body mass and triglycerides. In a cross-sectional study by Genovesi *et al.* (2008) on 5 to 11 year old school children, both BMI and waist circumference showed significant association to

the risk of hypertension after adjustment for age and sex. Similar research in Mexican American children aged 8 to 10 years showed waist circumference as the main factor associated with systolic blood pressure (Colin-Ramirez *et al.* 2009). Waist circumference is used as a marker for abdominal obesity and as an important component of metabolic syndrome (Genovesi *et al.* 2008), because in this study it is clearly associated with blood pressure and validated by other studies where it is associated with several chronic diseases including central obesity and insulin resistance (Goran *et al.*2003). The significant phenotypic correlation of SBP with all these traits results from the genetic effects but not environmental influences as shown by this study. This indicates that systolic blood pressure and other metabolic syndrome-related traits are under pleiotropic influences in these children.

When diastolic blood pressure is considered for bivariate genetic analyses, it shows similar genetic correlations with metabolic syndrome-related traits. This indicates that diastolic blood pressure, obesity (studies reported higher prevalence of hypertension in children with BMI $\geq 95^{th}$ percentile [Sorof and Daniels 2002]), fasting insulin, HOMA-IR, triglycerides, overweight, total fat mass, BMI and percent body fat have a substantial degree of pleiotropy i.e., additive genetic effects that are common to all of these traits. In Mexican Americans, metabolic syndrome-related phenotypes are influenced by a common set of genes in children and this finding is validated by several studies that involved Mexican American adults (Duggirala *et al.* 1996, 2001; Mitchell *et al.*1996). Thus, the clustering of MS-related traits due to the common genetic influences in adults is demonstrable in children of Mexican American ethnic background.

Although the bivariate analyses of SBP and DBP measures as continuous traits revealed significant additive common genetic influences on MS-related traits, the analysis of blood pressure as a dichotomous trait (i.e., elevated BP) failed to reveal similar patterns. This is in agreement with the general notion that the continuous traits are more powerful for genetic analysis compared to the dichotomized or discrete traits such as elevated BP and that the dichotomization of a continuously distributed trait such as blood pressure is associated with loss of power (Duggirala *et al.* 1997). Even though, the phenotypic correlations between elevated BP and other MS-related traits are found to be significant in this study, none of the genetic correlations are significant; perhaps this is due to dichotomization of the data which usually results in the decrease of power. However, an increase in sample sizes compared to the present data could address this issue efficiently. Environmental correlations in our study appear to be not significant, and the lack of strong random environmental correlations between the pairs of examined phenotypes suggests that the basis of the observed phenotypic correlations are attributable mainly to shared additive genetic effects, especially phenotypic correlations between blood pressure and MS-related risk factors in particular, obesity, adiposity measures, triglycerides and insulin levels.

Sorof and Daniels (2002) demonstrated that obesity-related hypertension is affected by insulin resistance, abnormalities in vascular structure and function and several other factors. Several studies have recognized the possible contribution of insulin resistance to hypertension, lipid disorders, and glucose intolerance (Cambien *et al.* 1987; Reaven 1988; DeFronzo and Ferrannini 1991; Ferrannini *et al.* 1991),

while others documented that the insulin variables load on the same factor as obesity and glucose variables, and sometimes lipid variables (Miegs 2000; Arya *et al.* 2002b). The HOMA-IR phenotype, a surrogate or indirect measure of insulin resistance based on information from fasting insulin and glucose has been frequently used in children to assess insulin resistance (Huang *et al.* 2002). Therefore, as found in this study, it is obvious that HOMA-IR (or insulin resistance) appears to have shared phenotypic and genetic effects with other traits related to metabolic syndrome. Similar conclusion was reached by Lin *et al.* (2006). In a study by Cruz *et al.* (2002), while examining blood pressure and its phenotypic relation to body composition and insulin sensitivity in black and white children, they found that insulin sensitivity was significantly associated with systolic blood pressure after adjusting for total fat and total lean mass, but not with diastolic blood pressure. However, previous studies have shown that in adults, decreased insulin sensitivity and hyperinsulinemia were correlated with blood pressure (Ferrannini *et al.* 1987; Berenson *et al.* 1997). The data depicting a clear relationship between insulin measures and blood pressure in children are scarce and only few represent this kind of epidemiological studies. Therefore, this study showed blood pressure as a continuous trait exhibited significant genetic correlations with HOMA-IR, fasting insulin, BMI and triglycerides, and is in agreement with some previously conducted research (Duggirala *et al* 2000a; Arya *et al* 2002a, 2002b).

The variance components approach used in this study assumes multivariate normal distribution; therefore, the issue of non-normality regarding the examined MS-related quantitative traits should be addressed. For this reason, outliers that are ±

4 SD from the mean for a given trait were not considered for the analysis, and certain variables were log-transformed. After accounting for the significant covariate effects (age, sex, puberty, physical activity and fitness) of a given trait, the residual kurtosis is within normal range for all the variables considered as assessed by the computer program SOLAR. It is reassuring to note that the two types of bivariate genetic analyses, where the blood pressure is considered either as a continuous trait or a dichotomous trait, generate similar patterns of phenotypic relationships between blood pressure and MS-related traits. A similar conclusion was reached by Burke *et al.* (2000) while analyzing acanthosis nigricans, a discrete phenotype, and its association with diabetes-related phenotypes. This indicates that even though continuous traits are more informative, but sometimes dichotomous traits also contribute to the understanding of the severity of the disease for study comparison, future algorithm construction and mapping efforts.

SAFARI data with other San Antonio family data

To assess the extent of MS-related burden in children compared to adults, the already established MS profiles for the parental cohorts of the SAFARI children can be helpful. Therefore, some of the major findings from San Antonio Family Diabetes Study (SAFDS), San Antonio Family Heart Study (SAFHS), and Veterans Affairs Genetic Epidemiology Study (VAGES) [given in Appendix 1] that have relevance to the present study were compared (Courtesy: Duggirala). The data from these studies for comparison should be interpreted with caution since the SAFDS and VAGES families were ascertained on probands with T2DM. In contrast, the SAFHS families

were randomly ascertained without paying attention to any disease condition. The prevalence rates of metabolic syndrome in SAFHS baseline data (FHS1), SAFDS baseline data (FDS1), and VAGES are 26%, 44%, and 53%, respectively. The MS profiles reflect the fact that these study samples are dominated with individuals afflicted with MS or its components. When the heritabilities of individual components of MS and their related phenotypes of the SAFARI data are compared with the San Antonio family data (see Appendix 2) (Courtesy: Duggirala), it is evident that the metabolic-syndrome phenotypes are highly heritable (especially for SBP [70% vs. 31%], DBP [70% vs. 27%], BMI [66% vs. 46%], waist circumference [66% vs. 43%], and albuminuria [49% vs. 30%]) in Mexican American children compared to the adults. The San Antonio Family Heart Study was designed to examine the genetics of heart disease and its risk factors in Mexican Americans who are considered at high risk for diabetes prevalence (Mitchell *et al.* 1996). The high heritabilities observed in Mexican American children (especially SBP and DBP) from the present study were confirmed when compared to the SAFHS (see Appendix 3). The heritabilities of SAFHS to SAFARI participants were: 18% vs. 70% for SBP, 28% vs. 70% for DBP, 39% vs. 54% for total cholesterol, 40% vs. 78% for triglycerides, 46% vs. 69% for HDL, 40% vs. 49% for LDL, 18% vs. 27% for fasting glucose, 16% vs. 25% for 2-hr glucose, 35% vs. 45% for fasting insulin, 42% vs. 66% for BMI. These data suggest that the clustering of the MS traits and their genetic bases are demonstrable even in children who are aged only 6-17 years.

It should be noted that heritabilities differ during an individual life span (e.g., genotype by age interactions) and also among populations because of different genetic make-ups and sharing of environmental risk factors. However, despite these caveats, the present data when compared to those in adults signify the increasing risk due to metabolic syndrome-related traits in Mexican American children, and many of the components of metabolic syndrome are already present in these children by about 11 years of age. Although SAFARI children come from the same low-income neighborhoods and families as of the adults, these children are at increased disease risk due to the nature of familial aggregation of the quantitative traits in contrast to the population-based data profiles. Some of the previous findings on Mexican American families support the idea of extending this study to localize major susceptibility genes influencing variation in blood pressure and other metabolic-syndrome related traits in children. For example, chromosome 6q influences insulin resistance and obesity-related phenotypes in Mexican Americans (Duggirala *et al.* 2001). This finding was further verified by a study by Meyre *et al.* (2004) in French families and has been replicated by a number of studies. The chromosome 7q11.23 is linked with the MS in SAFDS (Duggirala *et al.* 1996; Lehman *et al.* 2004); 7q31.3 is linked with the MS in SAFDS (Duggirala *et al.* 1996); 15q is linked with triglyceride concentration, mean arterial pressure and albuminuria in the SAFDS (Duggirala 2000b; Bhandari *et al.* 2002; Lehman *et al.* 2002). It would be interesting to examine whether such findings in adult cohorts have any relevance to the ongoing epidemic of childhood obesity and MS in children.

CHAPTER SIX: CONCLUSION

Although there are increasingly compelling data that metabolic syndrome and its components have strong genetic determinants, there are minimal data on the prevalence, nature, age of onset, and natural progression of metabolic syndrome-related abnormalities and their genetic basis in children, especially in Mexican American children. The nature of the relationship between blood pressure and metabolic syndrome has not been adequately explained. Given that the Mexican American population has the highest age-adjusted prevalence of metabolic syndrome, this study was performed on children to establish the precursors of the metabolic syndrome. A number of metabolic syndrome risk factors including hypertension, dyslipidemia, insulin resistance and microalbuminuria were analyzed in this study. This study provided an opportunity to verify whether the prevalence of metabolic syndrome-related traits such as hypertension, microalbuminuria, waist circumference, fasting glucose, and fasting insulin were similar between children and adults of Mexican American population. This study demonstrated high and significant heritabilities for the blood pressure, as well as for independent and individual components of the metabolic syndrome such as elevated blood pressure, microalbuminuria, insulin resistance, lipid, and body composition measures. There is evidence that disorders such as obesity, dyslipidemia, hypertension, insulin resistance, and microalbuminuria cluster to constitute the metabolic syndrome. Such patterns of clustering of correlated traits are partly due to pleiotropy (see Figure 2).

Blood pressure and metabolic syndrome-related trait variation in the Mexican American children are significantly different from children in other ethnic groups. The observed heritability estimates of these traits are higher in these children when compared to Mexican American adults. The relative contributions of both genetic and environmental influences were quantified, genes accounted for 25 to 85% (h^2; $e^2 = 1-h^2$) of the phenotypic variation in measures of blood pressure, adiposity, lipids, microalbuminuria, insulin, and glucose. With regard to age, gender, puberty, physical fitness and other environmental covariates accounted for <45% of the total phenotypic variance. These results also emphasize the importance of considering genetic factors in epidemiological studies of metabolic syndrome-related risk factors. As demonstrated by the bivariate analyses, the observed phenotypic correlations between the examined MS-related phenotype pairs (e.g., SBP and DBP with triglycerides, insulin and body composition) results from strong common genetic influences. For example, the total phenotypic correlation between these phenotype pairs is mainly contributed from genetic correlations (30-50%) and minimal is contributed by environmental correlation. Thus, pleiotropy may underlie the observed clustering of MS risk factors in Mexican American children.

An important contribution of this study is the identification of early signs or precursors of the MS in children, so that plans for early detection and prevention (e.g., behavioral intervention) of MS disease burden can be formulated and longitudinal studies can be performed to track individuals from childhood to adulthood in matters related to longitudinal risk for diseases such as T2DM and MS

(Steinberger *et al.* 2009). Another significant finding of this study is that the MS clustering pattern and its genetic basis found in adults is demonstrable in children, too. The next step of this research should be the identification of specific gene loci associated with variability of these traits by mapping efforts using techniques such as genome-wide linkage and association studies. The present study based on the information from SAFARI children provides an opportunity to compare the MS risk profiles of children and adults, and increases the proportion of genetic information to be extracted from the data (e.g., power to detect linkage). The results from this study facilitate the understanding of complex genetic and environmental influences as well as their interaction effects on variation in blood pressure measures in children. In addition, the potential common genetic or pleiotropic influences on blood pressure and its correlated phenotypes such as obesity are determined. Importantly, in addition to the observation that various MS-related in children have strong genetics determinants, these data demonstrated potential environmental influences (e.g., age, sex and their interactions, physical fitness and puberty) on blood pressure and other traits in the Mexican American youth. Ultimately, the findings from this study may contribute significantly to the development of effective strategies to prevent and treat children who are at high risk for hypertension and its co-morbid disease conditions.

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Appendix 1. Characteristics and prevalence of metabolic syndrome* among San Antonio adult family members participated in various studies** (unpublished data, Courtesy: Duggirala).

Phenotype	FHS1	FDS1	FHS 2	FGS	VAGES
N	1430	575	856	741	1395
Mean Age (years) ± SD	38.8 ± 16.8	42.4 ± 17.2	42.5 ± 16.1	44.3 ± 17.7	52.9 ± 15.2
Sex (% Female)	59.3	58.9	61.8	59.2	61.7
High Triglycerides (%)*	35.6%	48.2%	31.5%	37.5%	36.1%
Low HDL-C (%)*	37.9%	78.0%	46.5%	53.8%	77.8%
Abdominal Obesity (%)*	46.9%	50.7%	60.5%	61.4%	68.9%
High Fasting Glucose (%)*	17.4%	27.7%	25.8%	32.8%	78.0%
High Blood Pressure (%)*	27.8%	34.3%	37.9%	46.8%	54.2%
Metabolic Syndrome (%)*	26.4%	44.2%	35.9%	41.8%	53.3%

*MS according to NCEP/ATPIII definition; **FHS1 = SAFHS baseline, FDS1 =SAFDS baseline, FHS2 = SAFHS recall-1, FGS = SAFDS/SAFGS, and VAGES
San Antonio Family Heart Study (SAFHS), San Antonio Family Diabetes Study (SAFDS), San Antonio Family Gallbladder Study (SAFGS), Veterans Affairs Genetic Epidemiology Study (VAGES)

Appendix 2. Heritabilities of MS-related phenotypes: The San Antonio family-based studies (published and unpublished data, Courtesy: Duggirala).

Phenotype*	h^2 (%)	Study
Type 2 diabetes	53	SAFDS (baseline)
Insulin	53	SAFDS/SAFGS
Acanthosis nigricans	58	SAFDS/SAFHS
Fasting glucose	23	SAFDS/SAFGS
2-hr glucose	28	SAFDS/SAFGS
Adiponectin	30	SAFDS/SAFGS
Leptin	34	SAFDS/SAFGS
SBP	31	SAFDS/SAFGS
DBP	27	SAFDS/SAFGS
Waist circumference	43	SAFDS/SAFGS
Body mass index	46	SAFDS/SAFGS
Waist hip ratio	34	SAFDS/SAFGS
Alkaline phosphatase	27	SAFDS/SAFGS
ALT	18	SAFDS/SAFGS
AST	18	SAFDS/SAFGS
BUN	31	SAFDS/SAFGS
Bilirubin	48	SAFDS/SAFGS
Albuminuria	30	SAFDS/SAFGS
C-peptide	37	SAFDS (baseline)
IL-1β	60	SAFHS
IL-6	49	SAFHS
TNF-α	58	SAFHS
CCA-IMT	27	SAFHS
C-reactive protein	27	SAFHS
Birth Weight	68	SAFBWS

SBP = systolic blood pressure, DBP = diastolic blood pressure, ALT = alanine aminotransferase, AST = aspartate aminotransferase, BUN = blood urea nitrogen, IL-1β = interleukin-1β, IL-6 = interleukin-6, TNF-α = Tumor Necrosis Factor-α, CCA-IMT = common carotid artery IMT

Appendix 3. Components of Variance for Selected Cardiovascular Risk Factors from San Antonio Family Heart Study (Adapted from Mitchell *et al.* 1996).

Variable	n	Measured Covariates	Genetic	Household	Residual Environment
Lipids and lipoproteins					
Total cholesterol	917	.128‡	.392‡	.038	.442‡
HDL-C	916	.108‡	.455‡	.024	.413‡
HDL_3-C	916	.070‡	.375‡	.042	.513‡
HDL_{1+2}-C	916	.078‡	.368‡	.081†	.473‡
LDL-C	889	.092‡	.401‡	.074†	.433‡
ln(triglycerides)	917	.155‡	.396‡	0	.449‡
apoAI	807	.145‡	.431‡	.021	.403‡
apoAII	808	.136‡	.341‡	0	.523‡
apoB	807	.155‡	.308‡	.031	.506‡
apoE	805	.082‡	.333‡	.118‡	.467‡
LpAI	766	.177‡	.360‡	.118†	.345‡
ln(Lp[a])	906	.014†	.690*	.055	.241†
LCAT activity	672	.034‡	.206‡	.114‡	.646‡
Glucose and hormones					
Fasting glucose	869	.092‡	.183‡	0	.725‡
2-h glucose	850	.148‡	.159‡	0	.693‡
ln(fasting insulin)	785	.035‡	.348‡	.063†	.554‡
ln(2-h insulin)	765	.113‡	.129*	.086	.672‡
ln(SHBG)	677	.327‡	.126*	.031	.516‡
DHEAS	661	.323‡	.289‡	0	.388‡
Anthropometrics and blood pressure					
SBP	862	.308‡	.178‡	.081‡	.433‡
DBP	862	.146‡	.283‡	.050†	.521‡
BMI	949	.137‡	.424‡	.034	.405‡
WHR	942	.304‡	.058	.076‡	.562‡
STR	944	.309‡	.324‡	.012	.355‡

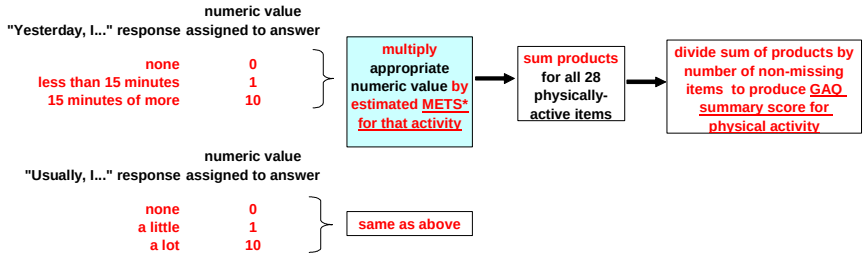
*P<.10, †P<.05, ‡P<.01.

Appendix 4: Calculation of physical activity and sedentary activity.

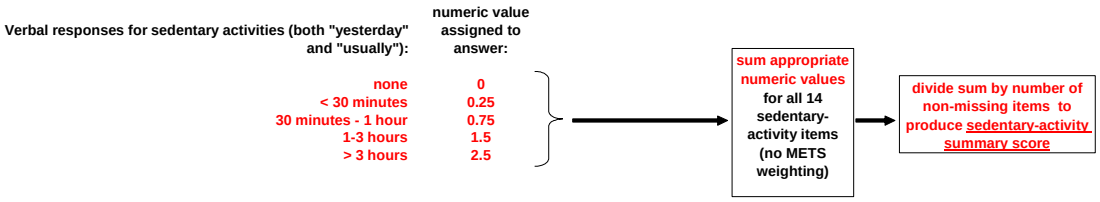
Girls health Enrichment Multi-site Studies (GEMS) Activity Questionnaire (GAQ)

(adopted for use as SAFARI physical activity recall)

How to analyze physical activity questions (items 1 - 28) to obtain summary physical activity score:



How to analyze sedentary activity questions (items 30 - 43), to obtain summary sedentary score:



For reference: Treuth et al 2003:534